INTERRELATIONSHIP OF NUTRITION, GROWTH, DEVELOPMENT AND INSECTICIDE EXPOSURE IN THE GERMAN COCKROACH (*Blattella germanica*) (L.)

BY

RICHARD DALE KRAMER

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1987

To Bonnie, Rachel and Joshua

and the memory of my mother,

Marjorie Caroline Kramer

ACKNOWLEDGEMENTS

The author wishes to thank the members of his committee for their assistance and support in the preparation of this work: committee chairman, Dr. Richard Patterson, who provided invaluable guidance and encouragement and afforded me the latitude to pursue my own objectives; Dr. Philip Koehler, who offered suggestions for allied research projects and assisted with statistical analysis; Dr. Frank Slansky who taught me the value and fun of ecology and whose Nutritional Ecology course provided me with the inspiration to do this study; and Dr. Michael Corbett, who graciously gave of his time to participate as an extradepartmental committee member.

Sincere appreciation is due to Mr. David Milne without whose technical laboratory assistance this research could not have been completed; to Mr. Troy Whitfield who provided guidance during toxicological studies; and to Ms. Margaret Martin who spent many long hours in manuscript preparation.

I would like to thank Dr. Carl Jones, Mr. Julio Hector, Mr. James Becnel, Mrs. Laura McDermott and Ms. Penny Cutler, all of whom contributed greatly to the success of this work. In addition to their valued technical assistance, expertise, and support, the friendship all of them provided has been immeasurable and long-lasting.

I am indebted to the United States Army which afforded me the opportunity to participate in the Longterm Civilian Training Program and to pursue this degree. Acknowledgement should be made also to the USDA Insects Affecting Man and Animals Research Laboratory for providing me the space in which to conduct my research.

I wish to thank my wife Bonnie for her support and encouragement during the past three years and for her assistance in the preparation and editing of this dissertation which greatly improved its quality. Finally, I am grateful to my children, Rachel and Joshua, for their understanding and patience during my indulgence.

TABLE OF CONTENTS

Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

INTERRELATIONSHIP OF NUTRITION, GROWTH, DEVELOPMENT AND INSECTICIDE EXPOSURE IN THE GERMAN COCKROACH (Blattella germanica) (L.)

By

Richard Dale Kramer

August 1987

Chairman: Dr. Richard S. Patterson
Major Department: Entomology and Nematology

Homeostatic processes within insects are affected by various extrinsic factors and are maintained through compensatory and/or inductive responses. A study was conducted to evaluate the interrelationship of two extrinsic factors (nutrition and pesticide exposure), on growth, development, feeding behavior and insecticide susceptibility in German cockroaches, Blattella germanica (L.).

Amount of dietary protein had no effect on susceptibility of male German cockroaches to propoxur and chlorpyrifos. The source of protein produced significant changes in insecticide susceptibility. Laboratory rodent chow increased total body lipids, carbohydrates and uric acid which may have resulted in increased tolerance to these insecticides. Three days of food and/or water deprivation

had little effect on chlorpyrifos susceptibility, but susceptibility to propoxur increased under these conditions. Following topical propoxur application, males lost 10% of their body weight in the form of water, but this loss did not result in significant changes in water or food consumption subsequent to treatment.

Hydroprene produced morphogenetic changes and increased total body mass in emergent adults. Morphogenetic changes in males occurred in the left and right phallomeres, subgenital plate and styli, precluding copulation and was related to dose and age of nymphal exposure. Total body mass of exposed males increased as much as 53% and of females as much as 47%; this change in body mass was accompanied by changes in water balance, total body carbohydrates, lipids and urates. Orlando normal males exposed to a low dose of hydroprene exhibited a slight increase in susceptibility to propoxur, while susceptibility to chlorpyrifos decreased. The multiresistant HRDC strain exhibited similar responses, except that a high hydroprene dose resulted in their increased tolerance to chlorpyrifos.

The compounds A9248 and allopurinol were evaluated as growth inhibitors. The antimicrobial agent, A9248, when formulated as a 4% bait, produced 100% mortality in nonchoice tests; in choice tests, however, 32% survived, and reproduction occurred. Allopurinol 2% bait inhibited the accumulation of uric acid in nymphs, reduced percent survival to between 2 and 7%, and postemergent adult

survivors failed to reproduce. Adults fed the bait produced significantly fewer nymphs per female.

CHAPTER I
THE GERMAN COCKROACH (Blattella germanica): A PERNICIOUS PEST OF HUMANS

Cockroaches are very primitive insects and are among the most pernicious pests of humans. Yet, of the thousands of known species of cockroaches, only a few are closely associated with humans, invading our homes, eating our food and transporting numerous disease-causing organisms. These few species of cockroaches have had ample opportunity to adapt their behavior in concert with humans through various selective pressures. Cockroaches have evolved numerous behavioral and physiological strategies which impact upon our ability to control these pests. Those few species of cockroaches which cohabitate with humans have been selectively pressured by extrinsic factors to evolve and survive our efforts to eradicate them. Many of these extrinsic factors center around the use of chemicals to control them and habitat modifications. These factors have severely limited cockroaches' ability to scavenge for food and necessitate the presence of adaptive mechanisms which permit them to undergo extended periods without food and/or water. The availability of water and a limited food source, such as protein, does not resolve this dilemma in the absence of other energy sources, such as carbohydrates and lipids.

The most persistent of the household infesting cockroaches is undoubtedly the German cockroach, Blattella germanica (L.), which has persevered despite all of man's efforts to eliminate it. This species has developed widespread resistance to a broad spectrum of insecticides. This resistance has been associated with both the presence of various endogenous detoxifying enzymes, and changes in behavior, e.g., avoidance, repellency, etc. With the continuous increase in the resistance of German cockroaches to insecticides, there has been a simultaneous decrease in effective control measures. It is, therefore, imperative that we develop a broader understanding of resistance mechanisms. Further, it is essential that we develop novel pest management strategies to deal with this pest in the future.

Since feeding directly affects all other behaviors in an insect's life and is critical to the perpetuation of the species, it is the most susceptible point. Although much is known about the nutritional requirements of the German cockroach, the relationship between nutritional status and pesticide resistance has not been investigated. Likewise, the effects of pesticide exposure on the growth and development of this species have been examined only cursorily.

The purposes of this study were to determine the interrelationship of nutritional status and insecticide tolerance in German cockroaches and to study the effects of

pesticide exposure on their feeding behavior, growth and development. The effects of nutritional status (water and food availability and various levels of dietary protein) on insecticide tolerance were investigated using carbamate and organophosphate compounds, propoxur and chlorpyrifos, respectively. The effects of the juvenile hormone analog (JHA), hydroprene, on growth, development and insecticide susceptibility were also investigated. Two additional compounds which affect food absorption and/or metabolic reserves were evaluated for their efficacy as control agents.

CHAPTER II
THE INTERRELATIONSHIP OF NUTRITIONAL STATUS AND INSECTICIDE TOLERANCE/RESISTANCE IN GERMAN COCKROACHES

Introduction

Slansky (1982) describes a paradigm of five key behaviors--survival, movement, reproduction, growth and feeding--which are essential for the perpetuation of a species. Feeding is the central behavior and facilitates all others. Feeding behavior may change as a result of inductive and/or compensatory responses to a variable environment. In order to achieve optimal performance, such responses may result in reduced fitness, e.g., increased susceptibility to pesticides.

Factors which may influence feeding behavior include deprivation, life cycle events, presence of members of the same or other species, temperature and the effects of other behaviors (Barton-Browne, 1975). Diet quality and quantity may also influence optimal growth. A holidic diet for xenic cultures of B. germanica was determined by Gordon (1959), and their essential nutritional requirements were compiled and reviewed by House (1961). Not surprisingly, these requirements were very similar to those of most insect species. Variation in the quantity and quality of various dietary constituents influences growth and development. For

instance, as the dietary protein level of German cockroaches is increased, their longevity decreases. Nymphal development and mortality are lowest on a 22-24% protein diet (Haydak, 1953).

Survival of many insects is predicated on the availability of metabolic reserves during periods of dietary stress. Many species of cockroaches have a unique metabolic reserve, uric acid, a by-product of protein catabolism. Originally considered storage excretion, it more recently has been categorized as a metabolic reserve utilized when a negative nitrogen balance exists in the diet (Cochran, 1985). Variations in dietary protein levels would, therefore, necessitate involvement of certain homeostatic processes.

Protein is an essential dietary component for most insects, however metabolic costs associated with its catabolism are high. The major by-product of its catabolism in insects, uric acid, complicates mass balance because there is less water associated with it; hence, the ratio of dry body weight to live body weight may significantly increase (Gordon, 1972). Engebretson and Mullins (1983) demonstrated that increased dietary protein increased the total body weight of German cockroaches. Tucker (1977a) reported that when urates were metabolized in Periplaneta americana fed on carbohydrate diets, K^+ was also released. Assimilation of nutrients in excess of requirements, such as high protein diets, requires increased excretion and

potential accumulation of toxic by-products (Gordon, 1959, 1972; Mullins and Cochran, 1975). Thus, protein availability and/or consumption presents an extrinsic stress which feasibly could increase susceptibility to pesticides. Lofgren and Cutcomp (1956) reported that P. americana on high protein diets were twice as susceptible to DDT. On the other hand, a 2.5% decrease in dietary protein in P. americana lowered its LD_{50} to malathion from 9 to 7 mg/kg (Appaiah et al., 1973). Perry and Agosin (1974) reported that milk-fed flies had elevated cytochrome P-450 as opposed to those fed sugar, and the LD_{50} for various insecticides was 1.5 - 2.3 times higher in milk-fed flies.

Just as quality and quantity of food might affect insecticide susceptibility, so might the depletion of metabolic reserves during dietary stress (starvation). The lack of food and/or water significantly reduces the longevity of B. germanica by approximately 90%; however, longevity of females fed water only was reduced by 50% (Willis and Lewis, 1957). Tucker (1977b) found that penultimate nymphs of P. americana, which have a larger lipid reserve than adults, were better adapted to surviving dehydration. The fat body of many insects serves as a "metabolic sink" for insecticides and their metabolites (Perry and Agosin, 1974). Hence, the quantity and quality of food, state of hydration and metabolic reserve of an insect could significantly affect susceptibility to insecticides.

Gordon (1972) stated that it would be difficult to produce a significant phenotypic change in a species by altering its dietary consumption. Several generations must be colonized on a new diet with relatively little change for its nutritive value to be determined (Dadd, 1970). Gordon (1972) concluded that polyphages must be a storehouse of genetic variability, protected by balanced polymorphism. This was demonstrated to a limited degree by Hampson and Steiner (1982), who correlated the extent of genetic variability in isolated German cockroach populations to insecticide exposure.

To assess the basic effects of nutritional deprivation, and, thus, the effects of metabolic reserve depletion on insecticide tolerance/resistance in this species, several studies were conducted. The impact of insecticide exposure on male cockroaches maintained on four different nutritional regimens (food and water, water only, food only and starved) was assessed using topical application techniques. Further studies were conducted to determine the effects of dietary protein levels on the metabolic reserves (water, lipids, carbohydrates and uric acid) of German cockroaches, and the subsequent impact this has on their susceptibility to two insecticides, chlorpyrifos and propoxur. The possibility of genetic drift due to long-term colonization on these three dietary protein levels (4, 20 and 50%) was also examined using starch gel electrophoresis.

Materials and Methods

The German cockroach strains used in this study were provided by the USDA Insects Affecting Man and Animals Laboratory, Gainesville, FL. The susceptible strain (Orlando normal), colonized in this laboratory since 1947, was used as the baseline in all studies. The multi-resistant field strain (HRDC) was a cross between a population collected from the U.S. House of Representatives in 1980 and a local population collected from the Gardenia Apartments, Gainesville, FL, in 1982.

Colonies were cultured in round or square galvanized wash tubs (ca. 57 l) fitted with a 10-cm-wide metal ring attached to the top of each container with masking tape. The underside of this flange and the inner top 10 cm of each tub were greased with a mixture of equal parts mineral oil and petrolatum to prevent escape. The bottom of each tub was lined with a sheet of heavy kraft paper which was secured using masking tape. Harborages were provided by rolling pieces of 10 x 15 cm corrugated cardboard and securing them with a rubber band. Each stock colony was provided food pellets (Purina Rodent Laboratory Chow #5001) and water ad lib. Water was supplied using gravity-flow chicken waterers with cotton strips in the trough to prevent free-flowing water. Colonies were maintained in separate rooms at ca. 27°C, 50% RH, and 8:16 hours (light:dark) photoperiod. Colonization of the HRDC strain varied in that

each generation was selectively pressured with diazinon and propoxur, by placing two plywood panels (15.24 x 15.24 cm) treated with 2.5 ml of a 1.0% acetone solution of each insecticide in the tubs after emergence of the second or third instar. The panels were removed after adult emergence.

Four dietary protein levels (0, 4, 20 and 50%) were used to study the effects of protein on insecticide tolerance in the Orlando normal strain. Colonies were maintained on their respective diets from initial eclosion and thereafter throughout each successive generation. These isocaloric diets were commercially produced by TEKLAD (Madison, WI), and varied primarily in their protein content (concentration adjustments were made with non-nutritive fiber and sucrose). A constant dietary fat level was maintained by adjusting the corn oil content to compensate for fat contributed by the casein. All other abiotic culturing parameters were unchanged.

The effects of water deprivation, food deprivation and starvation on insecticide tolerance were evaluated using the Orlando normal strain, while the effects of starvation were evaluated using the HRDC strain. Each treatment consisted of removing 250 cockroaches (1-7 days old) from the stock colonies and placing them in 9.57 l battery jars with cardboard harborages and the appropriate nutritional resources. To minimize control mortality and cannibalism,

the Orlando normal strain remained on this regimen for 72 hours; the HRDC strain remained on it for 144 hours.

In all other studies, male German cockroaches selected for susceptibility testing were 1-4 weeks old, since this age group does not differ in its susceptibility to propoxur and chlorpyrifos (Milio et al., 1987). Tolerance to propoxur and chlorpyrifos was evaluated using a topical application procedure adapted from Cornwell (1976) and Milio et al. (1987). Serial dilutions of both insecticides were prepared from technical grade material using acetone as a solvent. The dosage range varied, depending upon the cockroach strain and insecticide type. Adult male cockroaches were weighed in groups of 20, anesthetized with CO_2 and treated with 1 ul of toxicant on the first abdominal sternite, immediately posterior to the metathoracic coxae, using a hand-held Hamilton automatic 50 ul syringe. Groups of 10, 15, 20 or 25 specimens, depending on availability, were treated and then placed in 100 x 20 mm petri dishes. Due to excessive water excretion by those insects treated with propoxur, petri dishes were lined with Whatman No. 1 filter paper. Treated cockroaches were held for 24 hours at ca. 27°C and 50% RH, at which time mortality was recorded. Five insecticide doses, providing a 5% to 95% mortality range, were used for each experiment. All tests were replicated at least three times. Probit analysis was run on all data using the SAS statistical package (SAS Institute,

1979). Results were adjusted for control mortality using Abbott's formula (Abbott, 1925).

To assess the impact of these dietary regimes on German cockroach body constituents and nutritional reserves, the total body content of water, lipids, carbohydrates and uric acid were determined. Both male and female cockroaches 1-30 days old were removed from the colonies and stored at -60°C until analytical tests were performed. Total water content was determined by obtaining the wet weight of the cockroaches, then drying them to a constant weight (ca. 3 days) at 60°C and reweighing them on a Sartorius Model 2003 MP1 digital balance, accuracy ± 0.1 mg.

Total body lipids were determined by obtaining the wet weight of the specimen, macerating it in 4 ml of a hexane:ether solution (1:1 v/v) and allowing it to stand in a 23 ml sealed scintillation vial for 72 hours. The solvent containing the lipids was decanted, passed through a pasteur pipette packed with glass wool and collected in a predried/preweighed scintillation vial. The solvent was evaporated (24 hours), and the vial containing lipids was reweighed (Hector, J., unpublished data). This extraction method provided comparable results to a methanol:chloroform technique described by Williams et al. (1986) for ticks, but it was less time-consuming and could be used for individual analysis.

The anthrone reagent test described by Van Handel (1985) was used to determine total body carbohydrates. After the

wet weight of each cockroach was determined, the specimen was placed in a 16 x 100 mm glass culture tube and macerated in 1 ml of anthrone reagent (150 ml H_2O : 380 ml concentrated sulfuric acid : 750 mg anthrone). By adding more anthrone reagent, a total volume of 5 ml was obtained. The tubes were placed in an aluminum heating block (90 ± 2°C) for 17 minutes. A 1 ml aliquot of this reaction mixture was transferred to a clean tube, diluted to 10 ml with additional anthrone reagent and again heated for 17 minutes. The absorbance of this final solution was read against a standard anthrone blank using a Perkin Elmer Model 552 doublebeam spectrophotometer at a wavelength of 560 nm and a slit width of 4 nm. Percent absorbance was recorded 30 minutes after the final dilution, and total body carbohydrates were determined using a standard glucose calibration line. Total body carbohydrates were expressed as ug carbohydrate per mg wet body weight.

Extraction of whole body urates were made using modifications of the techniques described by Valovage and Brooks (1979) and Engebretson and Mullins (1983). Cockroaches were dried for 72 hours at 60°C, weighed and pulverized in the bottom of a glass test tube using a glass rod. Subsequently, 1 ml of 0.6% $LiCO_3$ (formulated in a 0.1 M glycine buffer, pH 9.4) was added to the dry material, and the reaction mixture was heated to 80 ± 2°C for 10 minutes. The sample was further diluted with $LiCO_3$ to 10 ml and then centrifuged at 1400 g for 10 minutes. To determine the

amount of uric acid present, 0.1 ml of the supernatant was transferred to a clean test tube containing 1.0 ml of Sigma glycine buffer (0.7 M, pH 9.4 at 25°C) and 6 ml water. After mixing, 3 ml of the solution was placed in a tube containing 50 ul water, and 3 ml was placed in a tube containing 50 ul uricase enzyme (0.4 units/ml : 1 unit converts 1 uM of uric acid to allantoin per minute at pH 8.5 and 25°C). These mixtures were allowed to incubate for 60 minutes and were then transferred to separate cuvettes. The difference in absorbance between uric acid and allantoin samples was determined using the previously described spectrophotometer (wavelength 292 nm and slit width 4nm). The absorbance difference was used with the dilution factor and the molar extinction coefficient of uric acid to calculate ug uric acid per mg dry body weight.

The mean total body mass, lipids, carbohydrates and uric acid were compared at different time intervals. The general linear models procedure using the Waller-Duncan method was used to separate significantly different means (SAS Institute, 1985). The minimum sample size in each replicate was 10 and varied, depending on the availability of specimens. All tests for significance were conducted at the $P = 0.05$ level.

To determine if there was any genetic drift in the Orlando normal population due to selection pressure induced by the various protein diets, allozyme analyses were performed using starch gel electrophoresis. The

electrophoresis techniques used in this study were adapted from Hampson and Steiner (1982). Four enzyme systems, ∝-glycerophosphate dehydrogenase (∝GPDH), esterase (EST), isocitrate dehydrogenase (IDH) and malic enzyme (ME) were run in a citric acid pH 8.0 (CA8) starch gel (gel buffer diluted 1:1) at 100v/50ma for 6 hours. Four other enzyme systems, glyceraldehyde-3-phosphate dehydrogenase (G-3PDH), phosphoglucomutase (PGM), phosphoglucose isomerase (PGI) and aconitase (ACON) were run in a CA8 gel (gel buffer diluted 3:1) at 100v/70ma for 6 hours. All electrophoretic and staining procedures were as described in or modified from Steiner and Joslyn (1979), except the staining procedure for ACON which was described by Harris and Hopkinson (1976).

Results

The effects of the various nutritional regimens on the tolerance of German cockroaches to topical applications of propoxur and chlorpyrifos were measured in terms of LD_{50} and LD_{90} and compared using a susceptibility ratio (lethal dose of normal population/lethal dose of the nutritionally deprived population). Thus, a higher susceptibility ratio indicated increased susceptibility of the nutritionally deprived population to that particular insecticide.

The topical application studies with propoxur (Orlando normal strain) as a function of three nutritional variables (water only, food only and starved) over a 3-day period are

presented in Table 2.1. The LD_{50} susceptibility ratios for propoxur did not differ significantly when comparing water only and food only (3.9 and 3.8, respectively). However, the starved population with a susceptibility ratio of 6.0 was 1.6x more susceptible than the other treatment groups. The pattern at the LD_{90} level was somewhat changed in that the susceptibility ratio for the treatment groups, food only and starved, were analogous, (5.8 and 6.6, respectively). However, the group on water only had a ratio of 2.0 which was approximately 3.2x less susceptible than the other groups. The slope of the log-dose probit (LDP) line for the latter treatment group is significantly flatter than all others and may account for this difference.

The effects of chlorpyrifos topical applications on treatment groups identical to those above are contained in Table 2.1. The 95% fiducial limits for all treatment groups except one did not overlap, indicating that a significant difference existed between their respective LD_{50} and LD_{90} values. However, the susceptibility ratios varied only slightly (1.1-1.3 and 0.9-1.1, respectively). The slopes of the chlorpyrifos LDP lines, though not identical, were relatively steep, indicating that the populations were homogenous.

The multi-resistant HRDC strain was used to compare the effects of topical applications of propoxur and chlorpyrifos on normally fed and starved treatment groups. The results of these tests are presented in Table 2.2. Because these

Table 2.1. Effects of 4 nutritional situations on the toxicological response of male Orlando normal German cockroaches.

Insecticide Nutritional Situation	n	Mean Wet Wt (mg)	Slope ± SE	SR[a]	LD_{50} (95% FL) (ug/g body wt)	SR	LD_{90} (95% FL) (ug/g body wt)
Propoxur							
Food and Water	600	47.9	1.02 ± 0.13	1.0	17.22 (13.15-30.06)	1.0	60.46 (33.3-484.07)
Water Only	425	44.5	0.67 ± 0.04	3.9	4.37 (3.85-4.89)	2.0	29.84 (24.55-38.42)
Food Only	575	38.4	1.56 ± 0.04	3.8	4.56 (4.38-4.74)	5.8	10.39 (9.77-11.15)
Starved	575	38.2	1.10 ± 0.07	6.0	2.85 (2.07-3.61)	6.6	9.19 (7.20-13.12)
Chlorpyrifos							
Food and Water	300	47.7	5.03 ± 0.22	1.0	4.42 (4.34-4.49)	1.0	5.68 (5.56-5.87)
Water Only	300	44.4	3.12 ± 0.14	1.3	3.33 (3.26-3.42)	1.1	5.02 (4.82-5.27)
Food Only	300	36.7	3.59 ± 0.16	1.1	4.14 (4.06-4.25)	1.0	5.94 (5.74-6.18)
Starved	300	37.6	2.41 ± 0.13	1.1	3.86 (3.72-3.96)	0.9	6.54 (6.20-7.02)

[a] SR: Susceptibility ratio = $\frac{LD_x \text{ Food and Water}}{LD_x \text{ Nutritional Situation y}}$

Table 2.2. Effects of 2 nutritional situations on the toxicological response of male HRDC German cockroaches topically treated with propoxur or chlorpyrifos.

Insecticide Nutritional Situation	n	Mean Wet Wt (mg)	Slope ± SE	RR[a]	LD_{50} (95% FL) (ug/g body wt)	RR	LD_{90} (95% FL) (ug/g body wt)
Propoxur							
Food and Water	425	55.7	0.81 ± 0.05	1.0	16.34 (15.17-17.77)	1.3	79.39 (62.89-107.56)
Starved	145	40.1	0.84 ± 0.08	0.3	4.88 (4.03-5.68)	0.4	22.30 (18.60-28.43)
Chlorpyrifos							
Food and Water	280	54.2	1.01 ± 0.07	13.4	59.37 (55.13-64.65)	37.0	210.41 (172.48-272.54)
Starved	260	39.6	1.59 ± 0.10	6.4	28.46 (26.72-30.10)	11.2	63.81 (59.09-70.08)

[a] RR: Resistance Ratio = $\frac{LD_x \text{ HRDC strain}}{LD_x \text{ Orlando normal strain fed food and water (See Table 2.1).}}$

treatments involved a comparison between a resistant and nonresistant strain, the conventional resistance ratio, resistant LD_x/susceptible LD_x (RR), was used for comparative purposes. The RR ratios at both propoxur dosage levels were similar for the normally fed HRDC and Orlando normal strains, while the starved HRDC treatment group was ca. 3x more susceptible at both dosage levels. These results were not expected, because the HRDC colonies were continuously exposed to residual applications of propoxur and were expected to have had a significantly higher resistance ratio. In calculating susceptibility ratios for the HRDC starved group and comparing them with the Orlando Normal starved group treated with propoxur (Table 2.1), it was found that they were elevated at both dosage levels (3.6:6.0 and 2.7:6.6, respectively). On the contrary, topical applications of chlorpyrifos, an insecticide which had not been used to pressure the HRDC strain, resulted in significantly different resistance ratios when comparing the normally fed groups at both dosage levels, 13.4 and 37.0. Starvation of the HRDC strain for 144 hours reduced the resistance ratio at each dosage level of chlorpyrifos to 6.4 and 11.2. Although lowered, the LD_{50} and LD_{90} of the starved HRDC group was significantly higher than the starved Orlando normal group, 28.46:3.86 and 63.81:6.54, respectively (Tables 2.1 and 2.2). The LD_{50} and LD_{90} values (dose expressed as percent) obtained by topical application for the Orlando normal (0.021 and 0.027%) and HRDC (0.32 and

1.13%) strains were comparable to those values reported by Milio et al. (1987) for the same strains (0.022 and 0.029%; 0.31 and 0.79%, respectively).

Changes in dietary protein levels of male German cockroaches did not affect susceptibility to propoxur and chlorpyrifos (Table 2.3). The 4, 20 and 50% casein diets produced no significant differences in the LD_{50} and LD_{90} propoxur values (10.71 : 11.12 : 9.46 and 35.60 : 37.10 : 66.80) or chlorpyrifos values (2.08 : 2.55 : 2.28 and 3.24 : 4.30 : 4.28). Larger differences were noted between those groups fed the laboratory rodent chow diet (23% crude protein) and those fed casein diets. The only exception was the higher LD_{90} value for the 50% protein group treated with propoxur, probably an artifact of the smaller slope for this LDP line. Cockroaches fed the lab chow diet and treated with propoxur were more tolerant (ca. 2x) than those fed casein diets at the lower dosage (16.15), while there was no appreciable difference at the higher level (37.74). This was also true of the lab chow fed group treated with chlorpyrifos with respective dosage values of 4.65 and 5.97.

The effects of the various diets on the total body constituents (water, carbohydrates, lipids and uric acid) of cockroaches 1-30 days old are presented in Table 2.4. There were few significant differences when comparing the wet weight, percent body water and dry weight of male cockroaches colonized on the casein diets; however, those fed a laboratory rodent chow diet had higher wet weights,

Table 2.3. Effects of 4 protein levels on the toxicological response of male Orlando normal German cockroaches.

Insecticide Protein Source	n	Mean Wet Wt (mg)	Slope ± SE	LD_{50} (95% FL) (ug/g body wt)	LD_{90} (95% FL) (ug/g body wt)
Propoxur					
4% Casein	289	48.2	1.07 ± 0.07	10.71 (9.00-12.28)	35.60 (29.23-47.39)
20% Casein	360	43.8	1.06 ± 0.05	11.12 (10.50-11.71)	37.10 (33.61-41.74)
23% Lab Chow	495	49.6	1.51 ± 0.04	16.15 (15.40-16.88)	37.74 (35.14-40.99)
50% Casein	345	44.4	0.66 ± 0.04	9.46 (8.67-10.23)	66.80 (54.75-86.24)
Chlorpyrifos					
4% Casein	195	48.1	2.92 ± 0.23	2.08 (2.02-2.14)	3.24 (3.10-3.43)
20% Casein	645	44.7	2.48 ± 0.38	2.55 (2.15-3.18)	4.30 (3.36-12.17)
23% Lab Chow	325	45.4	5.03 ± 0.22	4.65 (4.56-4.71)	5.97 (5.84-6.17)
50% Casein	300	45.1	2.05 ± 0.17	2.28 (2.20-2.37)	4.28 (3.99-4.72)

Table 2.4. Effects of 4 dietary protein levels on total body constituents of Orlando normal German cockroaches.

Sex Protein Source	Wet Wt (mg)	% Body Water	Dry Wt (mg)	Carbohydrate (ug/mg wet wt)	Lipid (ug/mg wet wt)	Uric Acid (ug/mg dry wt)
Male						
4% Casein	44.47b	68.52b	14.10ab	27.52a	66.00b	41.80c
20% Casein	43.42b	71.80a	12.25b	8.84c	71.60b	59.83c
23% Lab Chow	51.42a	67.10b	16.92a	15.12b	129.00a	161.08b
50% Casein	46.30ab	69.56ab	14.10ab	9.69c	50.20c	199.60a
Female						
4% Casein	104.00a	64.05ab	37.40a	20.35a	117.90a	33.40b
20% Casein	84.70b	65.21ab	29.50b	12.33c	133.20a	53.40b
23% Lab Chow	101.83a	63.70b	37.00a	17.56b	79.30b	123.92a
50% Casein	87.70b	66.65a	29.80b	16.42b	89.30b	104.40a

Means within a column (grouped by sex) followed by the same letter were not significantly different (P = 0.05; Waller-Duncan Method [SAS Institute, 1985]).

lower percent body water and increased dry weights, but, again, only a few of these differences were significant. Generally, lower wet weights were indicative of higher percent body water and consequently lower dry weights. Carbohydrates were highest in males fed a 4% casein diet followed by those fed laboratory rodent chow. There was no significant difference between those fed the 20 or 50% casein diets. Total body lipids were highest in males fed laboratory rodent chow, lowest in those fed 50% casein, and there was no difference in those on the 4 or 20% diets. Total body uric acid of males increased as their dietary protein level increased.

The water and dry weight relationship in females was similar to that of the males. The highest wet and dry weights of females were those fed 4% casein and lab chow diets, and while they were comparable, they differed significantly from those fed the other casein diets. Total body carbohydrates were highest in females fed the 4% casein diet and lowest in those fed 20%, while the values for those fed the 50% and laboratory rodent chow diets did not differ significantly from each other and fell between these two extremes. Lipids were highest for those fed 4 and 20% casein and lowest in those fed 50% casein and lab chow diets, although there was no significant difference within each pair. The results of female uric acid analyses were analogous to those of the males, except that females fed laboratory rodent chow had the largest amount, whereas, for

males, the highest uric acid concentration occurred on the 50% casein diet.

The percent cockroach survival on the 4, 20 and 50% casein diets was reduced significantly to 23, 34 and 30%, respectively, at the time of the first ootheca hatch, when compared to a 91% survival rate on the lab chow diet (See Figure 2.1). Development time from the first instar to emergence of the first adult was ca. 6 weeks for all four diets. An attempt was made to colonize this species concurrently on a 0% protein diet; however, percent survival was reduced to 6% at the time of first adult emergence (12 weeks), and only limited reproduction occurred.

Figure 2.2 is a diagrammatic representation of the electromorphs identified in the eight enzyme systems (GPDH, EST, IDH, ME, G-3PDH, PGM, PGI and ACON) used to determine if genetic drift had occurred. Regardless of diet type, no differences in the electromorph pattern of any enzyme system were detected.

Discussion

Tolerance of insecticides by cockroach populations may be affected by intrinsic behavioral (Rust and Reierson, 1977 and 1978; Lockwood et al., 1984; Pluthero and Singh, 1984; Brett and Ross, 1985), physical or biochemical resistance mechanisms (Terriere, 1982). Extrinsic factors such as temperature, humidity (Reichenbach and Collins, 1984),

Figure 2.1. Percent survival of German cockroaches fed varied protein diets as 1st instar nymphs. Results of linear regression analyses (slope ± SEM; r^2): ■ 0% casein (-6.70 ± 0.89; 0.72); + 4% casein (-6.58 ± 0.82; 0.76); ◇ 20% casein (-6.41 ± 0.65; 0.83); ▲ 50% casein (-6.23 ± 0.52; 0.87); x 23% laboratory rodent chow (-1.07 ± 0.12; 0.81).

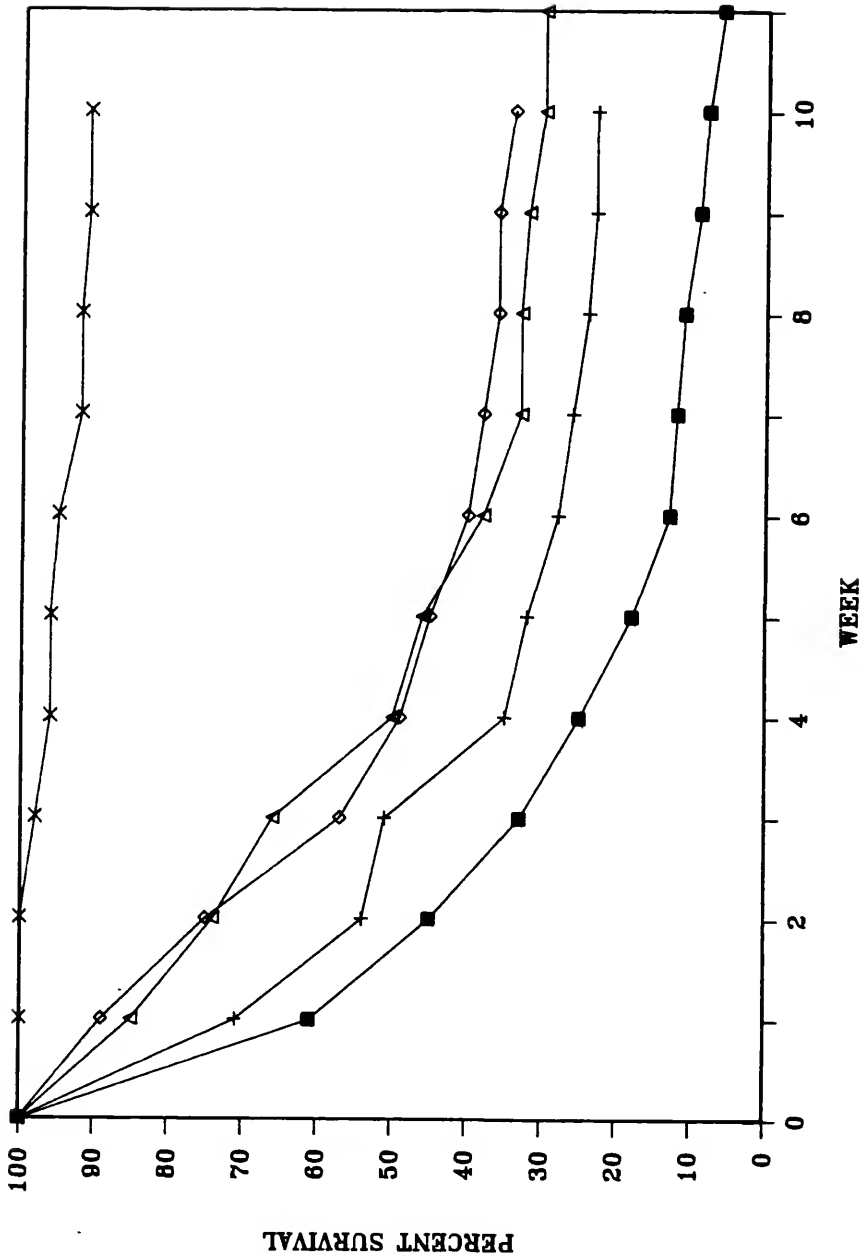
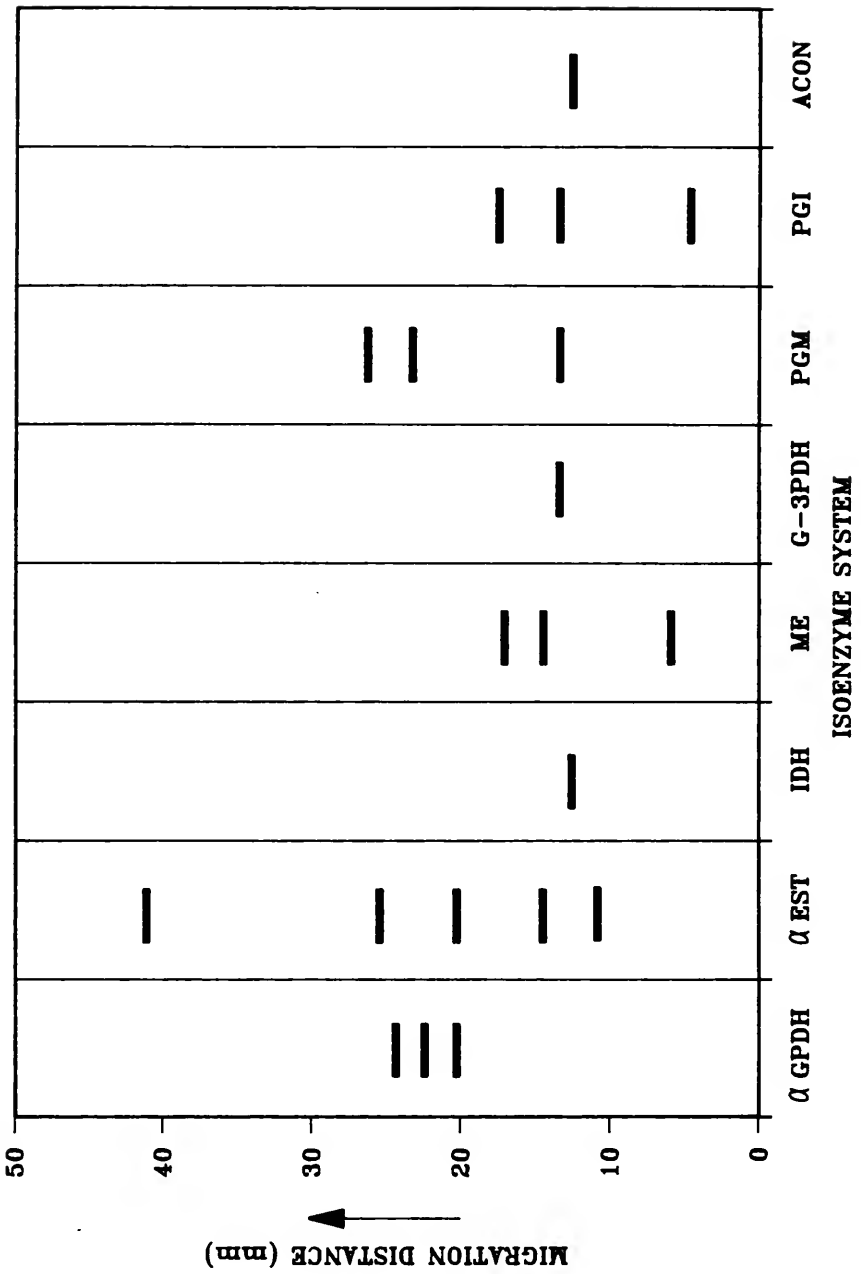

PERCENT SURVIVAL
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
WEEK

Figure 2.2. Electromorph migration distances for eight isozyme systems in male German cockroaches. Isozyme systems are: ∝ GPDH (∝ glycerophosphate dehydrogenase); EST (∝ esterase); IDH (isocitrate dehydrogenase); ME (malic enzyme); G-3PDH (glyceraldehyde-3-phosphate dehydrogenase); PGM (phosphoglucomutase); PGI (phosphoglucose isomerase); ACON (aconitase).


MIGRATION DISTANCE (mm)
0
10
20
30
40
50
α GPDH
α EST
IDH
ME
G-3PDH
PGM
PGI
ACON
ISOENZYME SYSTEM

crowding (Edwards, 1981) and the amount of protein in the diet (Lofgren and Cutcomp, 1956; Appaiah et al., 1973) may also affect resistance. Therefore, it would be reasonable to expect nutritional deprivation (of food and/or water) to enhance insecticide susceptibility. The data presented support this hypothesis, in that nutritional stress lowered the LD_{50} and LD_{90} values at all treatment levels.

The susceptibility of the Orlando normal strain to propoxur was affected to a greater extent than its susceptibility to chlorpyrifos where there was a minimal change in susceptibility. One factor which may have contributed to this effect is the water loss induced by topical applications of propoxur, a phenomenon not observed in individuals treated with chlorpyrifos. This will be discussed in further detail in Chapter III. Other factors that may account for this difference are cuticular penetration, differences in metabolic pathways, method of application, etc. (Terriere, 1982). Had the period of deprivation been extended for longer than three days, the effect undoubtedly would have been enhanced with both insecticides.

The effect of starvation on the resistant HRDC strain was comparable to the observations in the susceptible Orlando normal strain: an increase in susceptibility to propoxur of ca. 4x and 3x at the LD_{50} and LD_{90} levels, respectively. Susceptibility of starved HRDC cockroaches to chlorpyrifos increased ca. 2x and 3x at the same dosage

levels; however, their resistance ratios when, compared to the Orlando normal strain, remained elevated at 6.4 and 11.2.

Frishman (1982) and other pest management experts have stressed the importance of food and water elimination in cockroach control programs. The findings of Schneider and Bennett (1985) and this study, increased susceptibility to insecticides of cockroaches deprived of food and/or water, substantiates the importance of their recommendation.

Gordon (1961) hypothesized that insects colonized on any food that allows normal growth and development do not differ greatly in their degree of tolerance to contact insecticides. However, such broad generalizations are not always true, as demonstrated by Yu (1982) using the fall armyworm, Spodoptera frugiperda (J. E. Smith). He found that host plant variation induced changes in the larval microsomal oxidase activity, thus, changing their susceptibility to specific insecticides. Gordon (1959 and 1972) and Mullins and Cochran (1975) concluded that assimilation of nutrients in excess of requirements either by overeating or dietary imbalance required increased excretion and possible accumulation of toxins. This was the basis for the tentative hypothesis of this study: the depletion or accumulation of body reserves due to differences in dietary protein would produce nutritional stresses that would render this species more susceptible to xenobiotics. However, the results of this study, variation

in German cockroach dietary protein levels and source produced very little change (<2x) in their susceptibility to propoxur and chlorpyrifos, support Gordon's hypothesis. This was consistent with the studies of Lofgren and Cutcomp (1956) and Appaiah et al. (1973) on the effects of protein on the susceptibility of P. americana to insecticides. These findings of little impact of dietary protein variation on susceptibility to xenobiotics may be more typical of a polyphagous, opportunistic feeder, such as the cockroach, rather than more specific monophagous or oligophagous species which have coevolved with their natural hosts' nutrient levels and chemical defenses.

Changing the amount of protein in German cockroach diets had minimal impact on their percent body water, and although their wet weights varied as much as 18% and their dry weights as much as 38%, there was no apparent effect on their susceptibility to these two insecticides.

Although total body carbohydrates were 3x greater on the 4% diet and 2x greater on the laboratory rodent chow diet than the 20 and 50% diets, there was no apparent correlation to susceptibility. However, during periods of prolonged insecticide exposure or exposure to a chemical, such as propoxur, which induces excessive water loss (Chapter III), an increased carbohydrate reserve undoubtedly would provide an immediate source of energy and metabolic water via glycolysis.

Total body lipids were greatest in those male cockroaches fed the laboratory rodent chow diet, ca. 2x greater than those fed any of the casein diets. Each of the latter three diets had the same amount of dietary fat, and, thus, insects fed on them would be expected to have similar lipid reserves, providing consumption rates and assimilation efficiencies were similar. The laboratory rodent chow diet had approximately the same amount of lipid as the casein diets but consisted of crude and unbalanced sources of fat and protein as opposed to the refined and precisely balanced casein diets. The latter factor may have led to differences in the relative consumption rates of the various diets and, consequently, increased deposition of lipids in those insects fed the lab chow diets. Since the insect fat body is one of the two major organs involved in the production of detoxification enzymes (Terriere, 1982), the increased tolerance of the lab chow group (ca. 2x) possibly could be attributed to an increase in lipid deposition. The pattern of lipid accumulation in females was very erratic and was probably influenced by age variability (1-30 days), diet and degree of oogenesis. Valovage and Brooks (1979) related similar variability in uric acid deposition in this species to the same factors.

In this study, there was a specific trend in uric acid accumulation: as protein content of the diet increased so did deposition of whole body urates. The only exception was that females fed the laboratory rodent chow diet (23%

protein) had a higher uric acid content than those fed the 50% casein. Again, this may be attributed to the above-listed factors. Gordon (1972) stated that uric acid complicates mass balance since it has less water associated with it and may cause a significant increase in the ratio of dry weight to live body weight. This was not the case with German cockroaches used in the current study (i.e., the ratio varied only 5% in males and 3% in females). Originally, the accumulation of uric acid due to excessively high dietary protein levels was thought to be the major nutritional factor that would render the species more susceptible to insecticides. Several factors led to this hypothesis. Firstly, suboptimal amounts of a single nutrient can lower the use rate of others and decrease food utilization efficiency (House, 1961). Secondly, when urates are metabolized on low protein diets, some K^+ may be released, thereby disrupting the ionic balance and, possibly, producing nervous system dysfunction (Tucker, 1977a). Thirdly, both low and high dietary protein levels caused increased nymphal mortality and protracted nymphal development, while increased dietary protein shortened both adult and total lifespan (Haydak, 1953).

Tests conducted indicated that there may be a correlation between accumulation of whole body urates and susceptibility to propoxur and chlorpyrifos. Male cockroaches were slightly (<2x) more susceptible when fed on the casein diets (casein diets accounted for both the lowest

accumulation of uric acid at 4 and 20% and the highest at 50%). Uric acid accumulation in male cockroaches fed the laboratory rodent chow diet fell between these two extremes (See Table 2.4). These cockroaches were more tolerant of insecticide exposure, suggesting that there may be an optimal amount of stored urates with no deleterious effects on susceptibility. On the other hand, the quality of the diet, casein versus laboratory rodent chow, may have been a more important factor than uric acid level.

Data presented in Figure 2.1 indicated that the casein diet used in the study does not support optimal survival of this species, regardless of percent protein. This contrasted strongly with the work of Noland et al. (1949) and Noland and Baumann (1951) who reported optimal growth and survival on 30% casein, with lower and higher levels reducing both rates. With the exception of the 0% protein colony, colonies fed on the three casein diets had comparable percent survival rates, which were 3x less than the laboratory rodent chow colony. Despite this factor, the time of first adult emergence and of first ootheca hatch was approximately the same for all colonies, with the exception of the 0% protein colony. It was most likely that the meridic casein diet was deficient in some essential nutrient preventing optimal growth, since Gordon (1959) and the researchers listed above have successfully cultured this species on holidic casein-based diets. A reproducing colony has been continuously maintained in this laboratory on 0%

protein, since its origination with 300 ootheca in July 1985. Protein for this colony was presumably obtained via cannibalism.

Allozyme studies have been used by numerous researchers to identify sibling species and to provide evidence for genetic drift in isolated populations. These techniques were used by Hampson and Steiner (1982) to detect genetic drift in German cockroach populations which had been exposed to various insecticides and treatment regimens. Longterm colonization of this species with quantitative differences in dietary protein, as well as qualitative differences in protein source, failed to produce anomalies in the allozyme patterns of the enzyme systems studied. This was consistent with Gordon's (1972) prediction that major phenotypic characters show great stability over a wide range of environmental changes and that altering dietary composition would not produce a significant change in the phenotype of most species. This was also consistent with Ross and Cochran (1975), who, in studying genetic mutants of this species, found little genetic diversity.

An unexpected finding was that the resistant HRDC strain exhibited no resistance to propoxur, although it is continuously pressured with this insecticide and diazinon. Koehler (unpublished data) obtained similar results with propoxur when comparing mean time to mortality using a residue on glass test. The mean time to mortality for the Orlando normal strain was 0.35 hours, and the corresponding

value for the HRDC strain was 0.39 hours. These values were statistically different (P = 0.05; Waller-Duncan K-ratio T test [SAS Institute, 1985]); however, the resistance ratio (1.0) indicated no change in susceptibility. On the contrary, this strain was highly resistant to chlorpyrifos, a chemical which had not been used to pressure the colony. Factors possibly relating to this could be method of evaluating resistance, pressuring techniques, behavioral, physical and biochemical resistance mechanisms.

The repellency of propoxur (Brett and Ross, 1985) could account for this finding, because a small propoxur treated panel was placed in each large colony tub, but the cockroaches were not forced to cross it. Collins (1976), through selective pressure with propoxur, was able to increase the resistance ratio of German cockroaches to this insecticide 19x. This was achieved by confining nymphs to treated wooden panels (Collins, 1973), as opposed to a free choice method. Selection with propoxur also increased resistance to diazinon (14x) and chlorpyrifos (9x). The crossresistance induced by these compounds may account for the increased chlorpyrifos resistance ratio noted in this study.

This study dealt with the impact of nutritional stress on the accumulation or depletion of various body constituents and its subsequent effects on susceptibility to propoxur and chlorpyrifos. Further examination should assess the impact of such stresses on a population, since

the casein diets affect growth and longevity. The studies did not account for the nutritional costs of foraging, nor the effects of changes in consumption rates and assimilation efficiency with respect to insecticide exposure, all of which merit further study.

CHAPTER III
INDUCTION OF WATER LOSS IN GERMAN COCKROACHES BY SUBLETHAL DOSES OF PROPOXUR AND ITS SUBSEQUENT EFFECTS ON FOOD AND WATER CONSUMPTION

Introduction

Xenobiotics constitute a major threat to the perpetuation of insect populations and, therefore, cause a significant imbalance in the paradigm of insect nutritional ecology. Behavior provides an excellent avenue of adaptation to this threat, especially to temporally and spatially selective agents such as insecticides (Lockwood, 1984). Changes in behavior resulting from exposure to such compounds can induce changes in growth, reproduction, survival, movement and feeding as was demonstrated by Mansour (1978), using Spodoptera littoralis. He found that exposure of small larvae to low doses of three insecticides decreased body weight, produced lower weight pupae and lowered fertility, while exposing large larvae resulted in increased growth, increased pupal weight and increased fertility.

The effects of insecticide sublethal doses on German cockroaches have been studied by several authors. Sublethal exposure to DDT and BHC produced no significant differences in reproductive performance, although the number of nymphs

per ootheca and per female decreased in a DDT resistant strain (Grayson, 1951). Propoxur also reduced fecundity in exposed females (Riviere, 1977). Mansingh (1965) reported that malathion intoxication depleted amino acids, glycogen, trehalose and glucose due to enhanced activity of the Kreb's TCA cycle.

The behavioral and toxicological effects of insecticides on German cockroaches have been well documented and have been reviewed by Cornwell (1968) and Cochran (1982). This species has been noted to avoid surfaces treated with propoxur, diazinon and chlordane (Rust and Reierson, 1977 and 1978). Brett and Ross (1985) studied propoxur-induced dispersal in this species.

The availability of food and water to male German cockroaches has a significant impact on their longevity. Willis and Lewis (1957) reported normal longevity at 40% RH to be 54 days; but longevity was reduced by starvation to 8.2 days, food only to 8.8 days and water only to 9.6 days. Malathion exposure resulted in significant water loss (22.02%) in male German cockroaches through the spiracles and intersegmental membranes (Mansingh, 1965). Comparable water losses were detected in this laboratory when topically treating specimens with propoxur. A minimal amount of water would be contributed by catabolism of propoxur, since its detoxication in many insect species involves hydrolysis, N-dealkylation, O-dealkylation and ring-C-hydroxylation, resulting in the production of water and CO_2 (Perry and

Agosin, 1974; Matsumura, 1975). Reichenbach and Collins (1984) studied the effects of temperature and humidity on the susceptibility of German cockroaches to propoxur and found that susceptibility increased significantly at both high and low temperatures, and that low humidities increased toxicity at all temperature levels tested. The impact of sublethal insecticide exposure on German cockroach feeding behavior has not been studied. However, based on the consequences of such exposure and its impact on water and nutritional reserves, one could expect specific changes in feeding behavior.

Besides deprivation, many factors influence feeding behavior, including life cycle events, presence of other individuals, temperature and the effects of other behaviors (Barton-Browne, 1975). The purpose of this study was to assess an additional factor (sublethal propoxur exposure) which might affect feeding behavior in German cockroaches. The amount of weight (water) loss resulting from sublethal propoxur exposure and its effect on food and water consumption was evaluated over a 6-day period.

Methods and Materials

An initial study was conducted to determine the effects of propoxur exposure on water loss in male German cockroaches under various nutritional regimes. Males (1-7 days) were preconditioned for 72 hours on one of the

regimens (normal, water only, food only and starved) and then topically treated with a 1.0 ul solution of propoxur in acetone (0.1, 0.5 or 1.0 ug/ul). Controls were treated with acetone only. Treated specimens were transferred to a preweighed petri dish (100 mm x 20 mm) lined with Whatman No. 1 filter paper and the weight of the group (n=10 or n=25) was determined by reweighing. Posttreatment water loss was obtained by weighing each group of CO_2 anesthetized insects two hours after exposure and verified by reweighing their respective petri dishes and filter papers. The percent water loss was determined by dividing the wet weight of each group (two hours posttreatment) by the pretreatment wet weight. Mean percent water loss data was compared after arcsine transformation using the Waller-Duncan method (P=0.05, [SAS Institute, 1985]).

The effects of sublethal propoxur exposure on food and water consumption was determined using 1-7 day-old adult male German cockroaches from the Orlando normal colony. Cockroaches were only anesthetized for propoxur application, thus, reducing the dissolution of CO_2 in body water and minimizing the increase in wet body weight due to its excessive absorption, as recommended by Gordon (1972). Fifty adult males were transferred from the colony to a 3 l holding jar and were individually captured in a preweighed 23 ml screw cap vial and reweighed to determine individual pretreatment wet body weight. Each cockroach was then anesthetized, treated with a 1.0 ul solution of propoxur in

acetone (0.8 ug/ul) and transferred to a petri dish lined with filter paper, as described above. Individuals were held without food and water for 24 hours at which time they and their feces were transferred to preweighed vials and reweighed to determine the amount of water lost subsequent to the propoxur treatment. Those cockroaches which survived the propoxur exposure (ca. 50%) were used for food and water consumption studies. Ten cockroaches treated with acetone were used as controls for each replication.

Cochran's (1983) and Waldbauer's (1968) methods for determining the water and food consumption of individual female German cockroaches were adapted for this study. Because of the cockroaches' insensitivity to yellow and gold light (Koehler, et al., 1987), petri dishes were replaced with plastic amber snap cap bottles (110 cc) which eliminated the need for harborage within the container. Water bottles were fabricated by filling a 5 ml serum vial with water and capping the bottle with a rubber self-sealing septum through which the tip of an 18 gauge needle had been inserted, leaving the large end of the needle in the bottle. A cotton wick which was threaded through the needle extended 5 mm beyond the needle tip. The tip and exposed wick was inserted through a hole in the bottom of the insect holding chamber. The water bottles were weighed and then reweighed after a 2-hour preconditioning period (inserting the wick of the water bottle into an empty treatment chamber) to determine the amount of water loss due to evaporation alone.

Preconditioning was performed daily on each water bottle throughout the treatment period. Because there was excessive water loss due to evaporation over extended time periods, water was provided daily for 2 hours after the preconditioning period. Daily water consumption was determined by subtracting the water loss during the preconditioning period (after adjustment based on control water loss during the treatment period) from the water loss (evaporation and/or consumption) during the treatment period.

Laboratory rodent (mouse) chow pellets were dried at 60°C and were then placed in plastic caps. The food and container were weighed and were then placed in the holding chamber. Food consumption was calculated every 24 hours by reweighing the food and container and determining the change in weight after adjusting for changes in control weights.

Water and food consumption were monitored over 6 and 4-day periods, respectively, at which time the final weights of the cockroaches were determined and compared to the pretreatment weights. Food and water consumption data were analyzed using the Waller-Duncan K-ratio T test, $P = 0.05$ (SAS Institute, 1985).

Results

The interrelationship of nutritional status (3-day preconditioning period) on water loss and propoxur treatment

is presented in Table 3.1. Males provided food/water and water only had the highest mean percent water loss at the 1.0 ug/ul dosage level (9.87 and 7.80%) and the 0.5 ug/ul dosage level (10.03 and 9.23%), while the percent water loss was significantly different from the respective controls (1.60 and 1.83%). At the 0.5 ug/ul dosage level, mean water loss in groups with food only or starved was reduced (4.36 and 4.30%) when compared to the previously mentioned regimens. However, the mean water loss of these latter two groups was not significantly different from the control group (1.50 and 2.77%). The mean water loss (1.57 and 2.77%) in cockroaches treated with 0.1 ug/ul (food only and starved) did not differ significantly from their respective controls. The amount of water loss reflected the state of hydration in each treatment group.

The consequences of sublethal exposure (0.8 ug/ul) of male German cockroaches to topical applications of propoxur are presented in Table 3.2. The 24 hour posttreatment mean weight losses differed significantly among the three treatment groups, treated dead (10.25 mg), treated survivors (7.56 mg) and controls (4.45 mg). The mean weights regained at 6 days posttreatment were significantly different treated survivors (4.74 mg) and controls (2.52 mg). The percent weight regained for each of these groups were similar, 63 and 57%, respectively.

Food consumption was difficult to quantify; there was a wide range of variability (0-9.0 mg/day), largely due to

Table 3.1. Effects of the nutritional status of male German cockroaches on percent water loss, 2 hours after topical application of propoxur.

Nutritional Status	Percent Water Loss			
	Food/Water	Water Only	Food Only	Starved
Dose (ug/ul)				
1.0	9.87a	7.80a	-	-
0.5	10.03a	9.23a	4.36a	4.30a
0.1	-	-	1.57a	2.77a
Control	1.60b	1.83b	1.50a	1.73a

Means within a column followed by the same letter were not significantly different (P = 0.05; Waller-Duncan Method [SAS Institute, 1985]).

Table 3.2. Consequences of sublethal propoxur exposure on male German cockroaches.

Day Posttreatment	1	2	3	4	5	6
Treated[a] (n = 18)						
Weight Loss (mg)	7.56a	-	-	-	-	-
Weight Gain (mg)	-	-	-	-	-	4.74a
Consumption (% consuming)						
Food (mg)	2.32a (61)	0.37a (33)	0.82a (83)	0.36a (61)	-	-
Water (ul)	12.45a (100)	8.30a (100)	2.30b (67)	3.43a (78)	4.95a (94)	5.67a (94)
Controls (n = 19)						
Weight Loss (mg)	4.45b	-	-	-	-	-
Weight Gain (mg)	-	-	-	-	-	2.52b
Consumption (% consuming)						
Food (mg)	1.67a (53)	0.31a (26)	0.86a (84)	0.40a (37)	-	-
Water (ul)	10.38a (100)	6.97a (100)	6.84a (100)	4.72a (95)	6.73a (100)	6.33a (100)

Means (categorized by measurement) followed by the same letter were not significantly different (P = 0.05; Waller-Duncan K-ratio T test [SAS Institute, 1985]).

[a] Mean posttreatment (1 day) weight loss (10.25 mg) in dead males (n = 30) was significantly different from other treatment groups.

significant changes in the control weights. Because of these difficulties, the feeding portion of this study was terminated 4 days posttreatment, and only mean values and standard deviations were calculated. Food consumption per day and percent males feeding were highest on days 1 and 3 posttreatment, but there were no significant differences on any of the 4 days regardless of treatment.

Although water consumption in treated survivors on days 1 and 2 posttreatment (12.45 and 8.3 ul) was higher than that of unexposed controls (10.38 and 6.97 ul), the differences were not significant. In both groups, 100% of the males consumed water the first two days. On day 3 posttreatment, the situation changed: the controls consumed significantly more water (6.84 ul) than the treated males (2.3 ul), and only 67% of the treated group drank. On day 4, the percent of the treated group consuming water increased slightly to 78%; otherwise, during days 4-6, there was no significant difference in the amounts of water consumed by either group or in the percent drinking.

Discussion

The effect of food and/or water deprivation was demonstrated in Chapter II, where it was reported that these conditions increased male German cockroach susceptibility to propoxur 3.8-6.0x. The purpose of this study was to determine if water loss subsequent to propoxur exposure

contributed to these changes and whether the associated water loss would affect feeding behavior in sublethally exposed individuals. Male German cockroaches exposed to malathion lost 22.02% of their body water when unligatured and 15.70% when ligatured, compared to 3.80 and 3.30% in unexposed controls. Most of the loss occurred through the spiracles and distended intersegmental membranes (Mansingh, 1965).

The percent water loss observed in hydrated males 2 hours after exposure to 1.0 and 0.5 ug/ul propoxur was less than that observed subsequent to malathion exposure (Mansingh, 1965); however his observations were made 6 hours posttreatment. The corresponding controls demonstrated percent water losses ca. 3x less than values obtained by Appel et al. (1983), i.e., 7.5 ± 0.29% at 2 hours; however, their work involved desiccation of dead males. The percent water loss differences noted between hydrated and dehydrated groups could be related to the depletion of hemolymph volume during dehydration (Tucker, 1977c) or to the loss of sphincter control and the loss of water directly from the digestive tract. Other factors, previously cited, involved spiracular transpiration and intersegmental water loss. It is unlikely that cuticular water loss is an important factor since the cuticular permeability of German cockroaches is one of the lowest reported (Appel et al., 1983). Cuticular permeability may be an important factor in the penetration of propoxur, a highly polar molecule like carbaryl and noted

for its rapid penetration of insect cuticle (Terriere, 1982).

The slight reduction in water loss at the higher concentrations could be attributed to the fact that a greater proportion of groups were killed outright with little knockdown effect. Thus, metabolic shutdown would occur shortly after exposure, thereby diminishing the detoxification reactions that normally would ensue. The by-products of these reactions (water and CO_2), therefore, would be reduced (Matsumura, 1975). On the other hand, low dosages (0.1 ug/ul) caused no appreciable water loss compared to the controls, since the metabolic rate and degree of xenobiotic catabolism is related to dose. In examining individual responses to propoxur, a similar trend in weight (water) loss was noted over a 24 hour period. However, the water loss in treated dead males was higher (10.25 mg) than in treated survivors (7.56 mg), possibly due to increased metabolism, loss of spiracle or sphincter control. In fact, death may have been due to desiccation, the consequence of the increased water loss. After 6 days posttreatment, treated survivors had regained 63% of their weight loss compared to the 57% weight gain in controls, indicating that a relative degree of homeostasis had been achieved.

Slansky (1982) stated that three possibilities, short of death, exist in responding to environmental inputs: maintaining the "status quo," compensatory and inductive

responses. This limited degree of homeostasis could have been accomplished through food and water consumption, the only compensatory variables available during this study, or through reduced metabolism, an inductory response. This study considered only the compensatory responses.

Food consumption was the first compensatory response examined in response to sublethal exposure of male German cockroaches to propoxur. Although food consumption of treated individuals (2.32 mg) was higher than that of the untreated controls (1.67 mg) the first day following exposure, there was no significant difference. Similar observations made on days 2-4 indicated that food consumption rate per individual decreased and that there was no significant difference between treated survivors and controls. The percentage of individuals consuming food on a daily basis was fairly consistent between treatments except for the fourth day when 61% of the exposed group and 37% of the control group consumed food. The study was terminated on the fourth day posttreatment because of difficulties in quantifying the consumption rate. Gravimetric methods for these determinations had been adapted from Waldbauer (1968) and Cochran (1983) to study the individual feeding response, but due to large changes in the control food weights small individual consumption rates were obscured. Presumably, the dried food absorbed moisture from the holding chamber since most changes in the control food reflected an increase in weight and equilibration was not achieved. In future

studies, such problems might be circumvented by studying group food consumption, by reweighing the food after a longer period of time or by redrying the food prior to reweighing, thus, obtaining the dry weight of food consumed.

Providing water bottles for 2 hours reduced the problem associated with excessive water loss over an extended period of time (Cochran, 1983). Daily water consumption in treated males was significantly higher after exposure (days 1 and 2) and then significantly lower on the third day, when compared to the unexposed controls. The percentage of treated males consuming water was lowest (67%) on the third day. On days 4-6 there was no significant difference in the individual rate of consumption in either treatment group, and there were very slight differences in the percentage of males consuming water. This seemed to indicate that a certain degree of homeostatic water balance had been achieved 4 days posttreatment. The percentage of unexposed males consuming water daily was 100% except for the fourth day when 95% consumed water. Most males consumed water on a daily basis, indicating a substantial requirement for this nutritional resource. This may account for the increased mobility and higher percent recapture of males reported by Owens and Bennett (1982) when they studied the movement of German cockroaches within and between urban apartments.

The frequency of water consumption in males underscores the importance of water in relation to food, as was previously demonstrated by Willis and Lewis (1957). The

males' apparent daily necessity for water would increase the metabolic costs associated with foraging activity and increase the risk of exposure to xenobiotics (propoxur) and their metabolic consequences. Analogous differences in drinking frequency of females were reported by Cochran (1983), i.e., most females forming an ootheca consumed water daily, whereas those with a formed ootheca did not drink for 6-7 days.

The reduction in the percent of treated individuals consuming water on days 3 and 4 could be attributed to several factors: increased consumption subsequent to exposure could have initiated a feedback mechanism which attenuated the drinking response; a period of metabolic recovery following propoxur exposure may have mitigated the foraging response and reduced water consumption; and the documented repellency of propoxur (Rust and Reierson, 1977 and Brett and Ross, 1985) could have deterred foraging activity (consumption) after initial water deficits were overcome.

Exposure of male German cockroaches to propoxur produced significant water loss at 2 and 24 hours posttreatment. This depletion resulted in increased water consumption for the first 2 days which then plateaued for the duration of the study. No significant differences in food consumption were noted due to problems associated with gravimetric measurements. However, there was an indication that food consumption may have been higher on the day

following propoxur exposure. The sensitivity of this species to water and/or food deprivation and the effects of these conditions on insecticide susceptibility were demonstrated in Chapter II, further substantiating the need for food and water elimination in an integrated pest management plan for German cockroaches, as previously stated by Cochran (1983).

CHAPTER IV
GROWTH, DEVELOPMENT AND SUSCEPTIBILITY OF GERMAN COCKROACHES EXPOSED TO HYDROPRENE

Introduction

In the mid-1970s, juvenile hormone analogs (JHAs), such as hydroprene, were reported to cause sterility in adult insects exposed as nymphs or larvae (Slama and Williams, 1966; Das and Gupta, 1974 and 1977; Nosec et al., 1977; Cristodorescu et al., 1978), but it required another 10 years to establish an effective marketing strategy for cockroaches because insect growth regulators (IGRs), such as hydroprene, have no toxic effect on adults or nymphs (Staal et al., 1985). The value of some JHAs is their ability to suppress population growth by causing permanent sterility in exposed nymphs (Hangartner and Masner, 1973).

JHAs produce many other effects on insect populations depending on the species, stage of development, dose, duration of exposure, and volatility (Staal, 1975; Vogel et al., 1979). They may reduce ecdysone titers (Masner et al., 1975), cause morphogenetic anomalies (Das and Gupta, 1974 and 1977), inhibit ecdysis (Hangartner and Masner, 1973), affect vitellogenin synthesis and oogenesis (Lanzrein, 1974) and substitute for absent endogenous juvenile hormone (Sroka et al., 1975).

Exposing German cockroach nymphs to the JHA, hydroprene, has produced specific and permanent morphogenetic, behavioral and physiological changes in the population. The modulation of endogenous juvenile hormone synthesis by hydroprene (Tobe and Stay, 1979) may have a direct effect on observed changes, including prolongation of nymphal life and formation of nonreproductive supernumerary instars (Staal et al., 1985). Intermediates formed in cockroaches do not attempt to molt again, continue to feed and usually live longer than their reproductive counterparts (Staal, 1986). Three other differences between hydroprene-treated and untreated cockroach populations are often noted. Hydroprene exposure usually results in the formation of twisted wings and darker body coloration in emergent German cockroach adultoids, morphogenetic indicators of sterility. The last and most important difference is that treated populations fail to reproduce (Patterson and Koehler, 1985; and Staal, et al., 1985). Patterson and Koehler (unpublished data) found during cross-mating studies (normal males with exposed females and normal females with exposed males) that females fail to produce oothecae. Presently, there is no evidence to support the theory that reproductive failure following hydroprene exposure is due to true sterility alone.

The purpose of this study was to determine whether exposure of German cockroach nymphs to hydroprene induces morphogenetic anomalies in the male and female genitalia

which would produce an impotent condition, thus precluding successful copulation and subsequent reproduction. In addition, the effect of hydroprene dose and the age of nymphal exposure on the expression of these anomalies was evaluated. Total body mass, lipids, carbohydrates and uric acid were used to evaluate whether hydroprene exposure resulted in an unbalanced change in any of these constituents.

The practicality and effectiveness of combining residual insecticide and hydroprene treatments for German cockroach control were demonstrated by Bilbie and Nicolescu (1981) and Bennett et al. (1986). However, the interaction of these materials and their impact on susceptibility of German cockroaches to insecticides has not been investigated. Thus, toxicological studies with propoxur and chlorpyrifos were undertaken to evaluate the effects of increased body size and other factors associated with hydroprene exposure on the susceptibility of two strains of German cockroaches, susceptible (Orlando normal) and multiresistant (HRDC).

Materials and Methods

Dose-related effects of hydroprene on susceptible (Orlando normal) and multiresistant (HRDC) German cockroaches were studied using two different colonization techniques. Galvanized rectangular tubs (ca. 61,000 cm^3) or

glass utility jars (ca. 9,000 cm^3) were used to colonize 5,000 or 200 German cockroaches, respectively. The bottom of each metal tub was lined with brown kraft paper, and the upper lip was greased to prevent escape. Cardboard harborages, a one-quart gravity flow waterer and laboratory rodent chow were placed in each tub. The food and water were placed in the center of unpainted plywood panels (15.24 x 15.24 cm) which had been treated with 2.5 ml of 0.1 percent hydroprene (1.0 ug/cm^2). Either of two treatment regimens were utilized, 3 panels or 6 panels per tub. After placement of the panels, approximately 5,000 first instar nymphs were placed in the tub.

First instar nymphs were continuously exposed to hydroprene after being placed in glass utility jars, set-up using four cardboard harborages, a large plastic cotton-plugged water bottle and laboratory rodent chow, the latter two items were centered on a treated plywood panel. Panels were treated with 2.5 ml of 0.001, 0.005, 0.01, 0.05 or 0.1% hydroprene in acetone (equivalent to 0.05, 0.1, 0.5 and 1.0 ug/cm^2, respectively). Cockroaches were removed from tubs or jars for examination and testing after adult emergence, indicated by twisted wings (Patterson and Koehler, 1985).

The effect of hydroprene on growth and development was evaluated as a function of age, by exposing nymphal cohorts to 0.1% hydroprene treated panels. Treated panels were placed in jars containing 200 1st instar nymphs (1-2 d old) at 7-day intervals. Thus, the ages of initial exposure were

1-2, 8-9, 15-16, 22-23 and 29-30 days, and exposure was continuous until adult eclosion.

To ascertain the effects of hydroprene on the external genitalia of male German cockroaches, the following parameters were examined: expressability of the left and right phallomeres, size and position of both styli, and the shape of the left phallomere. Categories to describe the shape of the left phallomere were: 1 = normal, 2 = misshapened but possibly facilitates copulation, and 3 = undeveloped (nymphally preserved) and unable to be used in copulation (Figures 4.1-4.3). These physiological and morphogenetic anomalies were also correlated to the degree of wing twisting (TW0 = normal, TW1 = very tip of wing curled, TW5 = appear almost wingless, TW2 - TW4 = gradations of wing twisting based on the number of abdominal tergites covered) described by Patterson and Koehler (1985).

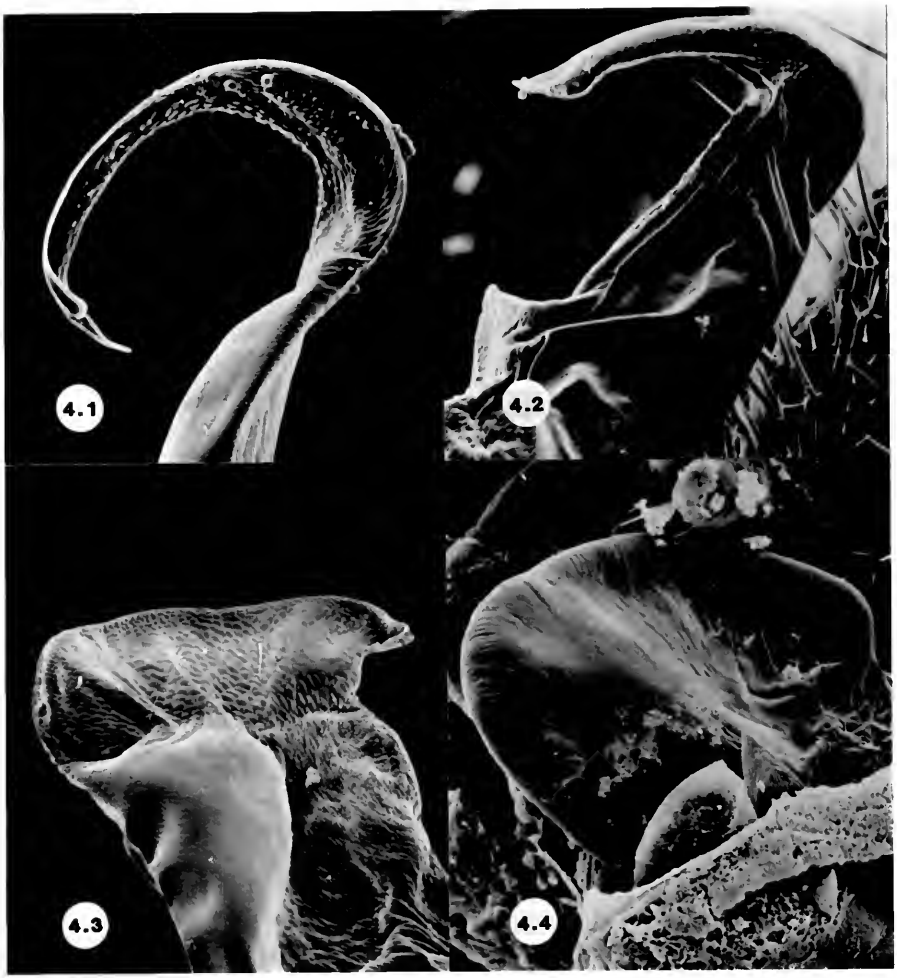
To study the morphogenetic abnormalities in more detail, live adults were removed and the external genitalia of both sexes were expressed with forceps and clamped with brass metal strips. Specimens were dehydrated in an ethanol series (70%, 80%, 95% and 100%), air dried (10 minutes), mounted on aluminum studs and sputter coated with gold. The internal female reproductive system was dissected in EDTA buffered saline, dehydrated in an ethanol series, fixed with hexamethyldisilazane (Nation, 1983), air dried (10 minutes), mounted and gold coated. To examine the external genitalia of male penultimate nymphs, the subgenital plate was removed

Figure 4.1. Left phallomere of a normal adult male German cockroach (1 = normal).

Figure 4.2. Left phallomere of a hydroprene-treated male German cockroach (2 = misshapened but possibly facilitates copulation).

Figure 4.3. Left phallomere of a hydroprene-treated male German cockroach (3 = undeveloped, nymphally preserved and unable to be used in copulation).

Figure 4.4. Left phallomere of an untreated penultimate male nymph with the subgenital plate removed.


4.1
4.2
4.3
4.4

prior to dehydration. All specimens were examined using a Hitachi S-450 scanning electron microscope (SEM).

To determine the effect of hydroprene on total body mass, both wet and dry weights of cohort adults (Orlando normal strain) were compared based on whether they were exposed or unexposed (mated and virgin) and on age of exposure (8-9 and 30-31 days), using the procedures described in Chapter II. Weight determinations based on age-related exposure were made using postemergent adults which were removed at weekly intervals for 4 weeks and then at 8 and 12 weeks. Dose-related responses were measured at 30 and 60 days only. The effects of 5 hydroprene dosage levels on body mass were evaluated at 30 and 60 days. Total body lipids, carbohydrates and uric acid were measured and compared in individuals exposed to hydroprene and in those that were both unexposed and mated. Postemergent adults were analyzed at 30, 60 and 90 day intervals using techniques and statistical analyses outlined in Chapter II.

The consequences of hydroprene continuous exposure (0.1%) on German cockroach susceptibility/tolerance to propoxur and chlorpyrifos were evaluated using both the Orlando normal and HRDC strains. Cohort colonies of both strains were established in separate tubs, the only difference being that one colony of each strain was exposed continuously to hydroprene after molting to the third nymphal instar, while the other was unexposed. Two dosage regimens, 3 and 6 panels per tub, were evaluated. The

topical application procedures and probit analysis used to assess susceptibility/tolerance were identical to those described in Chapter II.

Results

Figures 4.1-4.4 compare the development of the left phallomere in normal male German cockroaches, hydroprene-exposed male adultoids (2 most frequent forms) and normal penultimate nymphs. It appeared that the development of the left phallomere of hydroprene-treated males was morphologically intermediate between that of a normal nymph and that of a normal adult (the hook of the left phallomere was not fully developed). However, the undeveloped phallomere of the hydroprene-treated male was able to be everted from the phallic pouch, whereas the phallomere of a nymph could not. The external genitalia of normal and treated adult males and of untreated penultimate nymphs are compared in Figures 4.5-4.7. Figure 4.5 represents the typical normal male external genitalia; Figure 4.6 represents the genitalia of a hydroprene-treated specimen and Figure 4.7 represents the genitalia of an untreated male penultimate nymph. Major differences were apparent in the shape of the left phallomere, in development of the "phallic groove" (left lateral margin of the subgenital plate--Sternite IX) and in the size and position of the two styli. Preservation of nymphal characteristics was also noted in

Figure 4.5. External genitalia of a normal adult male German cockroach. Cercus (c); left phallomere (lp); left stylus (ls); "phallic groove" (pg); phallic pouch (pp); right phallomere (rp); right stylus (rs); subgenital plate - IX sternite (sgp); tenth tergite (tX).


4.6

Figure 4.6. External genitalia of a hydroprene-treated adult male German cockroach. Cercus (c); left phallomere (lp); left stylus (ls); "phallic groove" (pg); phallic pouch (pp); right stylus (rs); subgenital plate - IX sternite (sgp); tenth tergite (tX).


c
lp
c
tX
pp
4.6
um

Figure 4.7. External genitalia of an untreated penultimate male nymph with the subgenital plate removed. Left phallomere (lp); left stylus (ls); right stylus (rs); tenth tergite (tX).


lp
rs
ls
4.7
50um

the size and position of the styli. The ability to express the right phallomere was significantly diminished in hydroprene-exposed individuals.

Table 4.1 correlates hydroprene dosage and twisted wing classification with malformation of the left phallomere, expressability of the right phallomere and styli normality. The lowest dosage tested (0.001%) produced some wing twisting, mostly TW1, all left phallomeres were normal, and the frequency of right phallomere expressability and percent normal styli were comparable to the frequency of twisted wings. At dosage levels of 0.005% and 0.01%, the frequency of wing twisting increased, ranging from TW1 - TW5, while the frequency of left phallomere malformations remained low at 10 and 7%, respectively. However, the frequency of right phallomere eversibility and normality of the styli was almost uniformly 0%. This was also true for the two highest dosages (0.05 and 0.1%) at which all specimens examined were categorized as TW5. Malformation of the left phallomere was more pronounced at both dosages, with 75 and 96% being classified as type 3, respectively.

The effects of nymphal age at time of hydroprene exposure on the expression of the characteristics above are detailed in Table 4.2. Exposure of German cockroaches to 0.1% hydroprene during the first three instars produced very few differences in the expression of these characters. Most (97%) left phallomeres were category 3, 3-7% of the right phallomeres were expressible, and none of the styli were

Table 4.1. Percent morphogenetic anomalies in male German cockroach genitalia as a function of hydroprene dose.

Dose	TW[a]	LP[b] 1	2	3	RP[c] Eversible Yes	No	Styli Normal Yes	No
None (n=112)								
	0	98	0	0	85	13	98	0
	1	0	0	0	0	0	0	0
	2	1	0	0	1	0	1	0
	3	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0
	5	0	0	1	0	1	1	0
0.001% (n=85)								
	0	53	0	0	31	12	53	0
	1	38	0	0	19	19	38	0
	2	7	0	0	0	7	7	0
	3	2	0	0	1	1	2	0
	4	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0
0.005% (n=87)								
	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0
	2	2	0	0	0	2	0	2
	3	9	1	1	0	11	0	11
	4	6	0	0	0	6	0	6
	5	73	4	4	0	81	0	81

Table 4.1. Continued.

Dose	TW[a]	LP[b] 1	2	3	RP[c] Eversible Yes	No	Styli Normal Yes	No
0.01% (n=86)								
	0	0	0	0	0	0	0	0
	1	6	0	0	0	6	0	6
	2	13	1	1	1	14	0	15
	3	25	2	0	0	27	0	27
	4	15	1	0	0	16	0	16
	5	34	1	1	0	36	0	36
0.05% (n=84)								
	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0
	5	0	25	75	0	100	0	100
0.1% (n=93)								
	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0
	5	0	4	96	3	97	0	100

[a] Twisted wing categories defined by Patterson and Koehler (1985).

[b] Left phallomere classification: 1=normal; 2=deformed but maybe capable of copulation; 3=deformed and nonfunctional.

[c] Right phallomere.

Table 4.2. Percent morphogenetic anomalies in male German cockroach genitalia as a function of nymphal age at time of exposure.

Exposure Age	TW[a]	LP[b] 1	LP[b] 2	LP[b] 3	RP[c] Eversible Yes	RP[c] Eversible No	Styli Normal Yes	Styli Normal No
1-2 days (n=93)								
	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0
	5	0	4	96	3	97	0	100
8-9 days (n=79)								
	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0
	5	0	3	97	3	97	0	100
16-17 days (n=70)								
	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0
	5	0	3	97	7	93	0	100

Table 4.2. Continued.

Exposure Age	TW[a]	LP[b] 1	2	3	RP[c] Eversible Yes	No	Styli Normal Yes	No
23-24 days (n=50)								
	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0
	5	2	8	90	34	66	0	100
30-31 days (n=90)								
	0	27	0	0	24	3	14	13
	1	1	0	0	1	0	1	0
	2	2	0	0	2	0	2	0
	3	1	0	0	1	0	1	0
	4	0	0	0	0	0	0	0
	5	23	35	11	26	43	17	52

[a] Twisted wing categories defined by Patterson and Koehler (1985).

[b] Left phallomere classification: 1=normal; 2=deformed but maybe capable of copulation; 3=deformed and nonfunctional.

[c] Right phallomere.

normal. Exposure in the fourth instar lessened the effect in that 2% of the left phallomeres were normal, 8% were category 2 and 90% were category 3. Right phallomeres were eversible in 34% of the males examined, but none of the styli were normal. Exposure during the first four instars produced adultoids with TW5 wings (100%); those exposed during the fifth instar were classified primarily as TW5 (69%), followed by TW0 (27%) and the remainder (4%) were TW1 - TW3. Expressability of the right phallomere was 54% for all categories, while normal development of the styli occurred in 35% of the males examined. It is important to note that reproduction occurred only in the latter treatment group.

The structures of the female external genitalia which were examined included the gonapophysis (inner, ventral and dorsal), valvifers and vulva. Although major differences were found in the external genitalia of males, none were noted in females. The internal reproductive system of treated and untreated females were also examined externally via SEM. The median and lateral oviducts, and the colleterial glands appeared normal, but oocytes within the ovarioles appeared to be dehydrated and atrophied in females that had been exposed to hydroprene as nymphs.

Tables 4.3-4.4 compare the mean live weight, percent body water and dry weight of hydroprene-exposed German cockroaches during the second and fifth instars to unexposed mated and virgin males and females at 1-4, 8 and 12 weeks

Table 4.3. Comparison of the total body mass of adult male German cockroaches exposed to hydroprene as nymphs, and unexposed virgin and mated males, 1-12 weeks postemergence.

Week Postemergence	1	2	3	4	8	12
Treatment			Mean Live Weight (mg)			
0.1% hydroprene						
8-9 days[a]	55.3a	58.4a	60.6a	61.3a	74.6a	79.6a
30-31 days[a]	50.6b	50.5bc	56.8b	60.3a	-	-
Unexposed						
Mated	44.7d	51.1b	51.0c	56.2b	59.2b	52.0c
Virgin	47.6c	48.2c	48.2d	52.0c	59.5b	66.5b
Treatment			Mean Percent Body Water			
0.1% hydroprene						
8-9 days[a]	67.0b	64.0d	65.6c	63.1b	58.9c	57.8b
30-31 days[a]	70.8a	65.8c	70.2b	66.4a	-	-
Unexposed						
Mated	70.0a	71.6a	65.2c	67.7a	62.1a	66.0a
Virgin	70.8a	69.0b	72.2a	66.4a	60.8b	54.2c

Table 4.3. Continued.

Week Postemergence	1	2	3	4	8	12
Treatment			Mean Dry Weight (mg)			
0.1% hydroprene						
8-9 days[a]	18.3a	21.1a	20.9a	22.6a	30.6a	33.6a
30-31 days[a]	14.5b	17.4b	17.0b	20.3a	-	-
Unexposed						
Mated	13.4b	14.5c	17.8b	18.2b	22.4b	18.3c
Virgin	13.9b	15.0c	13.4c	17.5b	23.3b	30.5b

Values within a column followed by the same letter were not significantly different (P = 0.05; Waller-Duncan Method [SAS Institute, 1985]).

[a] Age of nymphs exposed to hydroprene.

Table 4.4. Comparison of the total body mass of adult female German cockroaches exposed to hydroprene as nymphs, and unexposed virgin and mated females, 1-12 weeks postemergence.

Week Postemergence	1	2	3	4	8	12
Treatment			Mean Live Weight (mg)			
0.1% hydroprene						
8-9 days[a]	99.5a	108.2a	109.7a	121.1a	134.4a	145.7a
30-31 days[a]	82.3b	103.8ab	105.5ab	111.4b	-	-
Unexposed						
Mated	75.0c	99.5b	103.8b	100.4c	100.2b	113.6b
Virgin	84.7b	92.5c	94.8c	98.7c	100.4b	99.2c
Treatment			Mean Percent Body Water			
0.1% hydroprene						
8-9 days[a]	62.3c	63.1a	60.3c	59.3c	56.5c	56.0c
30-31 days[a]	67.2b	62.5a	63.9b	62.5b	-	-
Unexposed						
Mated	72.1a	62.7a	66.0a	67.1a	64.8a	67.1a
Virgin	65.3b	62.9a	59.0c	60.6c	62.8b	59.5b

Table 4.4. Continued.

Week Postemergence	1	2	3	4	8	12
Treatment			Mean Dry Weight (mg)			
0.1% hydroprene						
8-9 days[a]	37.8a	39.9a	43.6a	49.2a	58.3a	64.2a
30-31 days[a]	27.4b	39.0a	38.2b	41.2b	-	-
Unexposed						
Mated	21.5c	37.2a	35.3c	33.0c	35.2b	33.4c
Virgin	29.8b	34.4b	38.9b	38.8b	37.6b	46.0b

Values within a column followed by the same letter were not significantly different (P = 0.05; Waller-Duncan Method [SAS Institute, 1985]).

[a] Age of nymphs exposed to hydroprene.

after adult emergence. The live weights of males increased with age except for those unexposed and mated and showed a significant decrease between 8 and 12 weeks. Male live weights were greatest in the hydroprene-treated population regardless of age and were highest (79.6 mg) at 12 weeks, followed by unexposed virgins (66.5 mg) and unexposed mated males (52.0 mg). Although the mean percent body water was the highest during the first week postemergence and was approximately the same for all treatment groups, it decreased with age regardless of treatment. The percent body water at 12 weeks ranged from a high of 66.0% for mated males to a low of 54.2% for unexposed virgins, with hydroprene-exposed males at 57.8%. The mean dry weight of hydroprene-treated second instars during the first week postemergence was ca. 25% greater than all other treatment levels. All dry weights increased with age and at 12 weeks postemergence, dry weights were significantly different in all groups although hydroprene-treated and unexposed virgins were similar (33.6% and 30.5%, respectively). The mean dry weight of mated males was ca. 40% less than the other two groups (18.3%).

Variation in the mean live weights of females was very similar to that of the males (lowest during the first week postemergence, increasing with age, and the highest at 12 weeks for those treated with hydroprene). The one difference noted was that unexposed mated females continued to gain weight whereas the males did not. Percent body

water in females followed the same pattern, being highest (62.3-72.1%) during the first week and decreasing over the next 12 weeks (56.0-67.1%). The percent body water of exposed second instar nymphs and unexposed virgins were lowest throughout the period, while unexposed mated females had the highest value. The mean dry weight of the females was similar to the males (the mean dry weight of hydroprene-exposed second instars was ca. 26% greater than all other groups). Dry weights increased over the 12 week period with the only noticeable decline in mated females ca. 3-4 weeks (time of first ootheca production). The highest dry weight attained (64.2 mg) was in the hydroprene-treated second instars ca. 2x greater than unexposed mated females (33.4 mg); virgin females (46.0 mg) were between these extremes.

The effects of hydroprene dose on mean wet weight, percent body water and dry weight of male and female German cockroaches at 30 and 60 days are represented in Tables 4.5 and 4.6. Examination of these parameters in males at 30 days postemergence was indicative of the sporadic effects of various hydroprene dosages. At 60 days the wet weights increased with dosage from 59.2 mg (unexposed mated) to 74.6 mg (0.1% hydroprene). With most treatments, dry weights also increased; the same two treatment groups which had the greatest difference in wet weight also had the greatest difference in dry weight (22.4 mg and 30.6 mg, respectively). The percent body water ranged from 62.9% to 58.9% but had no distinctive pattern.

Table 4.5. Effect of hydroprene concentration on the mean wet and dry body mass and percent body water in male German cockroaches, 30 and 60 days postemergence.

Time Postemergence	30 days			60 days		
	Wet Wt (mg)	Body Water (%)	Dry Wt (mg)	Wet Wt (mg)	Body Water (%)	Dry Wt (mg)
Concentration						
0.1%	60.1b	65.7cd	20.6b	74.6a	58.9e	30.6a
0.05%	64.5a	65.6d	22.2a	66.1b	62.9a	24.6b
0.01%	57.5c	64.7d	20.3b	63.0c	60.3cd	25.0b
0.005%	59.5b	66.7bc	19.8b	62.6c	61.5bc	24.1bc
0.001%	56.7c	71.5a	16.2d	61.4cd	62.4ab	23.1cd
Unexposed						
Mated	56.2c	67.7b	18.2c	59.2e	62.1ab	22.4d
Virgin	53.6d	65.5d	18.5c	59.5de	60.8cd	23.3cd

Values within a column followed by the same letter were not significantly different (P = 0.05; Waller-Duncan Method [SAS Institute, 1985]).

Table 4.6. Effect of hydroprene concentration on the mean wet and dry body mass and percent body water in female German cockroaches, 30 and 60 days postemergence.

Time Postemergence	30 days			60 days		
	Wet Wt (mg)	Body Water (%)	Dry Wt (mg)	Wet Wt (mg)	Body Water (%)	Dry Wt (mg)
Concentration						
0.1%	112.0ab	62.2b	42.4b	134.4a	56.5e	58.3a
0.05%	116.3a	60.1c	46.5a	120.5bc	59.3c	49.1c
0.01%	113.8ab	57.4e	48.4a	123.1b	57.2de	52.7b
0.005%	115.2ab	58.8d	47.6a	125.2b	58.4d	52.2bc
0.001%	109.8b	63.2b	40.4b	115.4c	62.2b	43.8d
Unexposed						
Mated	100.4c	67.1a	33.0c	100.2d	64.8a	35.2e
Virgin	92.3d	62.0b	34.9c	100.4d	62.8b	37.6e

Values within a column followed by the same letter were not significantly different (P = 0.05; Waller-Duncan Method [SAS Institute, 1985]).

Hydroprene-exposed females regardless of dose had higher wet and dry weights than the two unexposed populations at 30 days postemergence; however, there was no distinctive pattern within the hydroprene-exposed groups. Percent body water was lowest in the hydroprene-treated group, ranging from 57.4-63.2%, comparable to that of unexposed virgins (62.0%) but significantly different from unexposed mated females (67.1%). This pattern was identical for the specimens examined at 60 days postemergence except that those exposed to the greatest hydroprene dosage had the highest wet weights (134.4 mg), lowest percent body water (56.5%) and the highest dry weights (58.3 mg); unexposed mated females had reciprocal values of 100.2 mg, 64.8% and 35.2 mg.

The effects of hydroprene on mean total body carbohydrates, lipids and uric acid at 30, 60 and 90 day intervals are detailed in Table 4.7. At 60 days post-adult emergence, total body carbohydrates of unexposed males peaked at 23.5 ug/mg wet wt, and at 30 and 90 days it was 15.1 ug/mg and 15.8 ug/mg respectively; carbohydrates in the hydroprene-exposed group increased at each respective time interval from 16.2 ug/mg, to 18.5 ug/mg to 20.2 ug/mg. On the other hand, total body lipids showed an opposite pattern; the lowest value (20.7 ug/mg wet wt) occurred at 60 days and the respective values at 30 and 90 days were 129.0 ug/mg and 74.9 ug/mg. Total body lipids in hydroprene-exposed specimens declined with age, from 65.0 ug/mg, to

Table 4.7. Comparison of mean total body constituents of hydroprene - exposed and unexposed German cockroaches at 30 day intervals.

Sex Treatement Age	Carbohydrate (ug/mg wet wt)	Lipid (ug/mg wet wt)	Uric Acid (ug/mg dry wt)
Males			
Unexposed			
30	15.1d	129.0a	161.1e
60	23.5a	20.7c	337.9c
90	15.8cd	74.9b	392.2b
Hydroprene 0.1%			
30	16.2cd	65.0b	216.6d
60	18.5bc	25.7c	389.5b
90	20.2b	24.9c	628.1a
Females			
Unexposed			
30	17.6b	79.3b	123.9d
60	17.9b	57.6c	201.6c
90	16.3b	73.8bc	235.3b
Hydroprene 0.1%			
30	21.5a	102.0a	188.9c
60	17.9b	56.3c	274.2a
90	16.7b	77.1b	266.4a

Means within a column (grouped by sex) followed by the same letter were not significantly different (P = 0.05; Waller-Duncan Method [SAS Institute, 1985]).

25.7 ug/mg to 24.9 ug/mg. Uric acid deposition increased with age in both treatment groups; however, the rate was much higher in those exposed to hydroprene, so that at 90 days unexposed males had 392.2 ug/mg dry wt and those exposed had 628.1 ug/mg.

Total body carbohydrates in females ranged from 16.3 ug/mg to 21.5 ug/mg wet wt; only the latter value for 30 day postemergent exposed females differed significantly. Lipid deposition was similar to the trend noted in males (higher at 30 than at 60 days and increasing again at 90 days). As with carbohydrates, the highest value (102.0 ug/mg wet wt) obtained for total body lipids was for 30 day postemergent exposed females and was significantly different from all other treatments. Uric acid deposition was analogous to males, increasing with age and accumulating more rapidly in the treated groups; the highest value attained was 274.2 ug/mg dry wt in treated females, at 60 days.

Male German cockroaches were exposed to hydroprene as third instar nymphs and were topically treated with propoxur and chlorpyrifos. The results of these toxicological studies are presented in Tables 4.8 and 4.9. Both Orlando normal and HRDC strains exposed to a low hydroprene dosage (3 panels/tub) were more susceptible to propoxur (3x and 2x) at the LD_{50} level, while at the higher hydroprene exposure (6 panels/tub), no appreciable difference was found. Orlando normal males exposed to the higher hydroprene dose exhibited increased tolerance to propoxur at the LD_{90} level;

Table 4.8. Effects of hydroprene exposure on the susceptibility of male German cockroaches, Orlando normal and HRDC strains, topically treated with propoxur.

Strain Treatment	n	Mean Wet Wt (mg)	Slope ± SE	RR[a]	LD_{50} (95% FL) (ug/g body wt)	RR	LD_{90} (95% FL) (ug/g body wt)
Orlando normal							
Unexposed	345	47.9	1.51 ± 0.07	1.0	16.70 (15.87-17.54)	1.0	39.04 (36.33-42.38)
Hydroprene I[b]	240	51.4	0.70 ± 0.06	0.3	5.64 (4.86-6.42)	0.9	35.02 (28.02-47.47)
Hydroprene II[c]	240	56.7	0.91 ± 0.07	1.1	18.34 (16.75-19.93)	1.9	74.07 (60.85-96.30)
HRDC							
Unexposed	140	51.5	0.62 ± 0.05	1.0	16.31 (14.17-19.22)	3.3	128.16 (90.49-206.02)
Hydroprene I[b]	190	52.9	0.96 ± 0.05	0.5	7.56 (6.81-8.32)	0.7	28.92 (25.14-34.40)
Hydroprene II[c]	180	64.1	0.95 ± 0.09	1.1	18.88 (16.69-22.15)	1.9	72.70 (53.51-112.64)

[a] RR: Resistance Ratio = $\frac{LD_x \text{ Treatment}}{LD_x \text{ Orlando normal strain (unexposed)}}$

[b] Three (3) treated panels per tub.

[c] Six (6) treated panels per tub.

Table 4.9. Effects of hydroprene exposure on the susceptibility of male German cockroaches, Orlando strains, topically treated with chlorpyrifos.

Strain Treatment	n	Mean Wet Wt (mg)	Slope ± SE	RR[a]	LD_{50} (95% FL) (ug/g body wt)	RR	LD_{90} (95% FL) (ug/g body wt)
Orlando normal							
Unexposed	300	46.4	5.04 ± 0.20	1.0	2.80 (2.80-3.02)	1.0	3.66 (3.66-3.88)
Hydroprene I[b]	480	50.7	2.67 ± 0.17	1.1	2.96 (2.76-3.16)	1.3	4.73 (4.34-5.92)
Hydroprene II[c]	240	62.8	4.21 ± 0.20	1.3	3.50 (3.50-3.66)	1.3	4.78 (4.62-4.94)
HRDC							
Unexposed	640	50.6	1.51 ± 0.05	15.9	44.47 (43.08-46.05)	28.3	103.75 (97.83-111.26)
Hydroprene I[b]	400	50.1	1.66 ± 0.14	10.2	28.54 (24.55-32.34)	17.0	62.28 (50.90-92.42)
Hydroprene II[c]	200	68.4	1.54 ± 0.21	22.3	62.57 (50.44-96.64)	39.2	143.42 (94.01-672.81)

[a] RR: Resistance Ratio = $\frac{LD_x \text{ Treatment}}{LD_x \text{ Orlando normal strain (unexposed)}}$

[b] Three (3) treated panels per tub.

[c] Six (6) treated panels per tub.

HRDC males exposed to hydroprene were more susceptible than unexposed males or males exposed to the lower hydroprene dosage. The Orlando normal strain topically treated with chlorpyrifos showed no appreciable difference in its susceptibility at either dosage level regardless of hydroprene exposure. The HRDC strain exposed to a low dose of hydroprene was more susceptible to chlorpyrifos than was the unexposed group; those exposed to the higher dosage were more tolerant at both the LD_{50} and the LD_{90} levels.

Discussion

The left phallomere is essential to copulation in adult male German cockroaches. The male uses this structure to grasp the female's genitalia and to achieve the false linear position. Once this is accomplished, the male uses his forked basal hooks to grasp the female's ovipositor, the spermatophore is then manufactured and passed to the female via the endophallus. The copulatory act requires 72-115 minutes (Roth and Willis, 1952; Cornwell, 1968).

The severe malformation of the left phallomere in hydroprene-treated males precludes successful copulation, and renders the males impotent. This factor alone, however, does not explain the total reproductive failure noted in hydroprene-exposed males, since at low dosages, (<0.05%) the left phallomere was infrequently (<10%) malformed, yet reproduction did not occur. The inability to express the

right phallomere, another genital structure involved in copulation, may be a more critical element in the reproductive failure of males, since it was 97-100% apparent at dosages greater than 0.001%. A dose-related response of this species to another JHA (JTL-1) was reported by Cristodorescu et al. (1978) who found a direct correlation between dosage and nymphal prolongation and/or adultoid emergence. The impact of other anomalies noted in the male external genitalia (size and position of the styli and the shape of the "phallic groove") on mating behavior are undetermined, although the frequency of the former was similar to that of the right phallomere's expressability. These latter factors may be no more than additions to the catalog of morphogenetic effects of IGRs which have been detailed by many authors (Das and Gupta, 1974 and 1977; Hangartner and Masner, 1973; Masner and Hangartner, 1973; Nosec et al., 1977; Patterson and Koehler, 1985; Staal, 1975).

With the exception of 30-31 day old nymphs, nymphal age at time of exposure to 0.1% hydroprene had no significant effect on the expression of the morphogenetic anomalies previously discussed (98-100% of the left phallomeres were deformed, 3-34% of right phallomeres were eversible, and none of the styli were normal). This contrasted with the findings of Nosec et al. (1977) who used a different JHA (JTC-1) and reported an increase in morphogenetic effects as nymphal age of exposure increased. Patterson and Koehler

(1985) reported that 0-4 day old nymphs and those within 0-4 days of adult eclosion were insensitive to a 2-hour hydroprene exposure, as indicated by absence of twisted wings. Staal (1986) reported that emergent adults and "adultoids" exhibiting morphological deformations due to hydroprene exposure were sterile. He stated that only those nymphs treated during a critical period, early in the last instar, would show permanent sterility. The results of this study (exposure of 30-31 day old nymphs resulted in ca. 50% of the emergent adults lacking twisted wings and the morphogenetic anomalies that would physically preclude copulation) were consistent with their findings. Limited reproduction would, thus, be expected in this treatment group as occurred in the current study. However, this study differed from those cited above in that a continuous exposure was used, and exposed 1-2 day old nymphs were classified as TW5 and were sterile.

There were no morphological anomalies in the female external genitalia that should have precluded mating. Furthermore, the SEM examination of the internal reproductive system failed to reveal any morphological differences in the oviducts or colleterial glands. The oocytes of hydroprene-treated females either failed to mature or degenerated, and although this may have been due to undetected morphological changes, it also could have been a physiologically-induced effect. Similar observations were

made by Das and Gupta (1974 and 1977) using three different JHAs.

Staal (1986) reported that treated nymphs upon reaching the "adultoid" stage no longer attempt to molt but, instead, continue to feed and may have extended longevity, well beyond that of reproductive adults. Based on the observation in this laboratory that hydroprene-exposed German cockroaches weighed 6-35% more (depending on postemergent age, sex and age at time of exposure) than their cohorts (Tables 4.3, 4.4, and 4.7), analyses were conducted to determine if a change in the relative proportions of certain total body constituents had occurred.

Hydroprene exposure resulted in significant increases in the live and dry weights of both males and females and in a corresponding reduction in the percent body water when compared to their mated and virgin counterparts. Although water differences were gravimetrically determined, the presence of atrophied oocytes are further evidence that IGR treated females suffer dehydration. Similar findings by Das and Gupta (1977) using different IGRs support these results. The increase in dry weight could be attributed, in part, to the accumulation of total body urates (Table 4.7), although the concomitant decrease in the percent body water could not be explained since uric acid formation is the most efficient method for maintaining nitrogen and water balance (Mullins and Cochran, 1983). A more tenable explanation for the increase in total body mass in hydroprene-exposed and

unexposed virgin cockroaches is the lack of metabolic costs associated with mating behavior, sperm/spermatophore production in males or oogenesis and ootheca production in females. These weight and percent differences were enhanced by increased hydroprene dosage and became more divergent with age. Endogenous juvenile hormone (JH) titers decline with age and life cycle events; however, this titer may be maintained artificially with exogenously applied JHA and this may directly affect total body carbohydrates, lipids and uric acid.

Allatectomy increased whole body glycogen in several insect species, due, presumably, to the subsequent cessation of protein synthesis and diminished need for (glycogen) energy, (Steele, 1983). Thus, the total body carbohydrates of German cockroaches, especially females, would not be expected to increase with age until the high energy demand reproductive cycle waned, after the fourth ootheca production at ca. 120 days (Willis et al., 1958; Cochran, 1983). Factors influencing this would include reduced titers of JH required for vitellogenesis and increased consumption of food associated with ootheca production. Female German cockroaches appeared to fit the predicted pattern (unexposed reproductives had a relatively constant carbohydrate level [30/60/90 days], whereas continuously hydroprene-exposed cohorts had a significantly higher initial level at 30 days which then declined over the remaining time period). Male German cockroaches remain

active as long as 100 days following adult emergence (Roth and Willis, 1952), and although this protracted sexual activity and its aforementioned metabolic costs may explain the changes observed in total body carbohydrates, it fails to justify the observed increase in total body carbohydrates of hydroprene-exposed males. An alternative explanation may be found by considering the significant quantities of total body urates in the treated group and the increased metabolic energy (glycogen) needed for its production (Lehninger, 1982).

Allatectomy also affects the accumulation of lipids, resulting in the hypertrophy of the fat body due to increased lipogenesis (Beenakkers, 1983). Thus, in the absence of JH, the storage function of this organ continues since amino acids and carbohydrates are available for conversion to lipids. Unexposed male German cockroaches showed a significant dip in total body lipids at 60 days postemergence with a subsequent increase at 90 days. This cyclic occurrence corresponded inversely with the change in total body carbohydrates which were higher at 60 days but which then declined at 90 days. These changes may be directly related to hormonal changes associated with the reproductive cycle. Hydroprene-exposed males exhibited a decline in total body lipids as a function of time, a result which might be expected if the regulatory mechanism for lipogenesis is sensitive to the exogenous application of JHA. Metabolic homeostasis in reproductive females was

evident throughout the treatment period with regard to total body lipids, as it was in carbohydrates (the levels were relatively constant during the 90 day period). Exposed females exhibited a lipid deposition pattern similar to that of treated males (an initially high level and then a decline in total body lipids), presumably for the reason previously stated.

Mullins and Cochran (1983), in their review of nitrogen metabolism in insects concluded that the corpora cardiaca and the corpora allata were involved in the regulation of fat body urate levels. Generally uric acid increases due to increased nitrogen intake, lack of an essential amino acid/starvation or increased K^+ concentration in the diet. Results of this study indicate that there is a continuous increase in total body urates after adult emergence, reaching a relative "saturation point" between 30-60 days in male and female German cockroaches, although accumulation is graded beyond this point. This contrasts with the findings of Valovage and Brooks (1979), who reported that males approached maximum levels of uric acid at 10 days postemergence, while females continued to accumulate uric acid up to 50 days. Their work differed from the present study in that they calculated the percent uric acid in the fat body, rather than total body urates. This finding may indicate that accumulation of uric acid may continue in other body tissues even though the fat body (urocytes) are replete. Hydroprene treated and unexposed females in this

study had deposition patterns similar to theirs, except accumulations leveled off 60-90 days postemergence and females exposed to hydroprene accumulated more uric acid. Males exposed to hydroprene typified the pattern Valovage and Brooks (1979) observed in normal females (a sharp, continuous increase in uric acid deposition up to 90 days). JH reportedly has a dual effect on fat body metabolism, suppression of lipid synthesis as previously discussed, and stimulation of protein synthesis. Thus, if hydroprene can induce the same events as JH (protein synthesis), one of the resultant by-products undoubtedly would be uric acid.

The evidence substantiates that hydroprene disrupts German cockroach metabolic homeostasis by affecting water balance, total body lipids, carbohydrates and uric acid, thereby creating a potentially stressed condition. The impact of two of these conditions, dehydration and increased total body urates, on the susceptibility of German cockroaches to propoxur and chlorpyrifos have been documented in Chapters II and III. This study concludes with an evaluation of the impact of hydroprene exposure and its consequences, as previously discussed, on the susceptibility of male German cockroaches to propoxur and chlorpyrifos.

Despite the physiological and morphological changes identified in adult and adultoid German cockroaches and induced by nymphal exposure to hydroprene, there was no significant difference in male susceptibility to the two

insecticides tested. However, the toxicological findings indicated that those males exposed to the lower hydroprene dosage were more susceptible to both insecticides, except for the Orlando normal strain which was slightly more tolerant of chlorpyrifos. Thus, in terms of cost and potential effectiveness, lower doses of hydroprene may be more efficacious and certainly warrant further investigation.

It is important to note that the previously discussed biological parameters were measured using specimens exposed to the lower hydroprene dosage. It would be easy to speculate that the increase in susceptibility was due to hydroprene's disruption of metabolic homeostasis; however, this would not account for the increase in tolerance noted at the higher dosage. Two questions remain: 1) do higher hydroprene dosages disrupt homeostasis to the same extent as lower dosages? In all probability, they do because there are significant differences when comparing the wet and dry weights and the percent body water of insects exposed to high and low hydroprene dosages. It would appear that disruption of these homeostatic processes (excessive accumulation of uric acid), have little impact on susceptibility to these insecticides (as was previously demonstrated in Chapter II). 2) do the innate mechanisms for JH degradation have any effect on the activity of these insecticides? This question awaits further research.

CHAPTER V
EVALUATION OF A9248 AND ALLOPURINOL, GROWTH INHIBITORS OF GERMAN COCKROACHES

Introduction

Growth inhibitors can function in numerous ways depending on the target organism, the mode of action and the particular compound itself. The specific responses of the target organisms may involve reduced or lack of reproduction, reduction in growth rate, reduced adult emergence and/or death. The interrelationship of insect nutrition and growth inhibition were discussed by Gordon (1961 and 1968) who stated that growth failure may be the result of any one of several factors: blockage of nutrient absorption at transfer sites; poor efficiency of conversion of absorbed food due to essential nutrient deficiency or blockage by antimetabolites; or poorly digested food due to enzyme inhibition by an antimetabolite.

Current research has focused on IGRs such as hydroprene (Staal, 1985), fenoxycarb (Brenner et al., in press) and alsystin (Weaver et al., 1984) all of which interfere with ecdysis and/or block reproduction. Few studies have identified and evaluated the efficacy of nutritional growth inhibitors for German cockroaches,

although the elimination of food and water to facilitate control in pest management has been advocated. Beck and Stauffer (1957) reported that 6-methoxybenzoxazolinone, a naturally occurring growth inhibitor found in corn reduced the growth efficiency of German cockroaches. Cycloheximide inhibited protein synthesis, thereby depressing growth, but it had no effect on oxidative metabolic weight loss. On the other hand, veratrine and cocaine severely depressed oxidative metabolic weight loss, while only veratrine depressed growth (Gordon, 1968).

The potential of two chemicals, A9248 and allopurinol, were evaluated as nutritional growth inhibitors for German cockroaches. A9248 (diiodomethyl p-tolyl sulfone) manufactured by Abbott Laboratories is an organic compound containing iodine and is used as an antimicrobial agent (mildewcide) in paint. This compound destroyed the termite hindgut protozoa, Trichomitopsis termopsidis (J.A. Breznak, unpublished data) and effectively controlled workers of the Formosan subterranean termite, Coptotermes formosanus (Su and Scheffrahn, unpublished data). Allopurinol is a structural analogue of the natural purine base, hypoxanthine, and is an effective inhibitor of xanthine oxidase, and, thus, blocks the formation of uric acid, the metabolic end product of purine catabolism in humans. However, it differs from uricosuric agents in that it does not lower serum uric acid levels in humans. German

cockroaches, as well as many other species, store large amounts of uric acid (Chapter II) either as a metabolic reserve or as a result of storage excretion. Cochran (1985) reviewed the interrelationship of three cockroach cell types (trophocytes, mycetocytes and urocytes) within the fat body and the ability of these insects to utilize stored uric acid during periods of nutritional stress. The deposition of paternal urates during copulation and their subsequent incorporation into the ootheca has been reported as an additional function of this metabolic reserve in German cockroaches (Mullins and Keil, 1980). Engebretson and Mullins (1986) reported that total body urates of German cockroaches decreased when adults were fed increasing amounts of allopurinol.

It was hypothesized that A9248 would affect the mycetocytes and allopurinol the urocytes, reducing the accumulation of uric acid, thus affecting survival and reproduction.

Materials and Methods

A9248, allopurinol and two uricosuric compounds, probenecid and sulfinpyrazone, were screened as baits for the control of German cockroaches. Tests were also conducted to determine the efficacy of A9248 as a topical and/or residual toxicant. Baits were formulated at various

concentrations (w/w) by dissolving or suspending (allopurinol) the appropriate amount of technical material in 15 ml of acetone and mixing this solution with a sufficient amount of powdered 20% casein or ground laboratory chow to make 25 g of bait. Control baits were formulated by mixing the dry bait material with acetone. Acetone was evaporated from the baits, which were then ground to a fine powder and stored at 4°C until used.

Preliminary studies were conducted by placing 50 third instar nymphs (17-24 days old) in a 1-gallon utility jar the upper lip of which was greased with a mixture of equal parts mineral oil and petrolatum. The jar contained two corrugated cardboard harborages, a plastic water bottle and a paper souffle cup filled with the appropriate bait. Tests were conducted at 25 ± 1°C, 50 ± 1% RH, and a photoperiod of 8:16 (L:D).

Mortality and the total group weight of all surviving cockroaches were recorded at weekly intervals. As adult emergence occurred, the number of males, females, nymphs and ootheca were recorded. Each test was replicated at least twice and was terminated when hatch or total mortality occurred.

A9248 was evaluated as a bait in both 20% casein and standard laboratory chow at concentrations of 2.0, 4.0, 8.0 and 12.0% (w/w). Replicates consisted of either 50 nymphs per 3.8 l jar or 100 nymphs per 7.6 l jar. Toxicity of

topically-applied A9248 was assessed at a 4.0% concentration using procedures described in Chapter II (3 replicates of 10 males each). Residual activity was evaluated by treating plywood panels with 2.5 ml of either a 1.0, 2.0 or 4.0% solution of A9248 in acetone. Panels were dried for 4 hours prior to cockroach exposure. Testing conditions were identical to those outlined in Chapter IV except a 20% casein diet was used instead of lab chow. A 4% lab chow bait was used to evaluate nymphal age response to A9248. Four nymphal stages (2nd-5th) were studied using 100 nymphs per replication. Choice tests, using 2nd instar nymphs, were conducted by placing two souffle cups in each treatment jar, one containing the standard laboratory rodent chow and the other containing a 4.0% A9248 laboratory rodent chow bait. Finally, a 1.0% A9248 laboratory rodent chow bait was tested in conjunction with a 0.1% hydroprene residual treatment to determine if the efficacy of either could be enhanced (see Chapter IV).

Allopurinol was evaluated as a bait in 20% casein, as well as standard laboratory rodent chow using a 2.0% (w/w) concentration. The effect of this compound on reproduction in 1-2 day old postemergent adults was studied by feeding unmated adults (25-30 females and 10 males) a 2.0 percent bait in 20% casein, either 72 hours or continuously. Like A9248, allopurinol was evaluated as a 2.0% bait in conjunction with 0.1% hydroprene residual treatments.

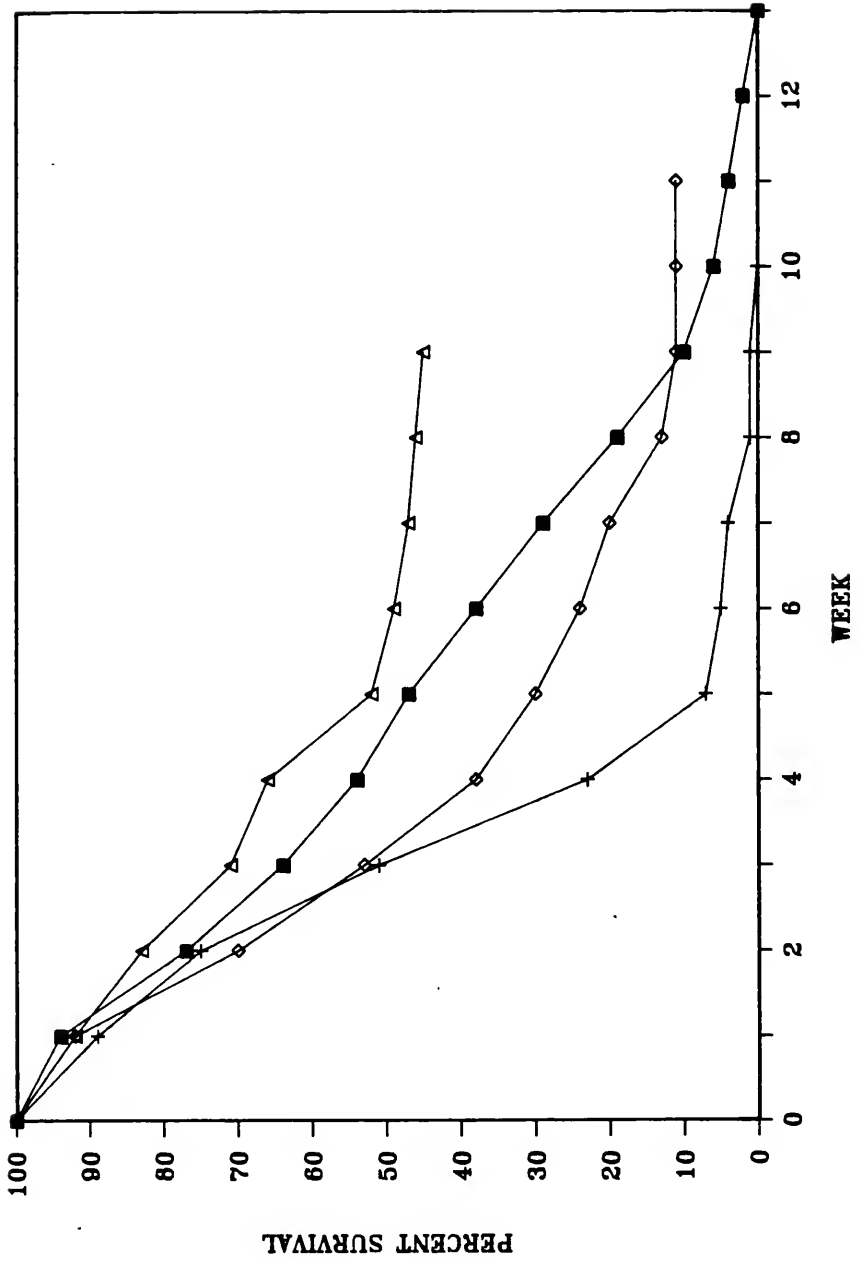
Choice tests were also conducted, as above, using a 2.0% bait in laboratory rodent chow. Two-week old mated adults (50 females and 50 males) were given a choice of 2.0% bait or untreated lab chow to determine if postemergence exposure would affect hatch. Linear regression equations were used to determine if the percent survival of treated populations/week was affected by exposure to A9248 or allopurinol.

Uric acid analyses, as described in Chapter II, were used to determine the effects of allopurinol on German cockroach total body urates. Analyses were performed on penultimate nymphs, emergent adults (1 day old), post-emergent adults and adults which had been continuously fed allopurinol bait during their development. Mean values were determined and analyzed for significance ($P = 0.05$) using the Waller-Duncan method (SAS Institute, 1985).

Results

Preliminary bait screening indicated that only A9248 and allopurinol had any deleterious effects on percent survival and reproduction. Probenecid and sulfinpyrazone, uricosuric compounds, had no apparent effect on these parameters. Figure 5.1 compares the effects of 2% allopurinol bait, 4% A9248 bait, 0% casein diet and the 20% casein control diet on the percent survival/week of 3rd

Figure 5.1. Screening test: Percent survival of cockroaches fed 2% allopurinol bait, 4% A9248 bait, 0% casein or 20% casein as 3rd instar nymphs. Results of linear regression analyses (slope ± SEM; r^2): ■ 2% allopurinol (-8.64 ± 0.33; 0.96); + 4% A9248 (-12.24 ± 1.13; 0.87); ◇ 0% casein (-8.32 ± 0.70; 0.86); △ 20% casein (-6.55 ± 0.41; 0.87).


PERCENT SURVIVAL
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
WEEK

instar German cockroach nymphs. The 0% protein diet was used as a comparative baseline to establish the consequences of no protein on percent survival. Data points were terminated at the week in which either reproduction or total mortality was noted.

In bait tests (no choice), nymphs fed the A9248 bait either failed to molt or molted only once prior to death. Total body mass of individuals increased 1.6x over the 10 week period prior to death, while the body mass of controls increased 5.1x. Nymphs fed allopurinol bait exhibited delayed and asynchronous adult emergence 5 weeks posttreatment and total mortality occurred during week 13. Controls had a more synchronous emergence at 4-5 weeks; however, survival on the 20% casein diet was reduced to 45%. The percent survival of the population fed 0% casein at 5 weeks was 30%, while survival in the allopurinol population was 47% and in the A9248 population was 7%. In contrast to the treated populations, the 0% casein population had emergent adults which produced 1 viable ootheca. A similar study was conducted on 2nd instar nymphs and produced comparable results, except that percent survival was reduced ca. 6%, all A9248 treated nymphs died by 9 weeks and longevity of allopurinol and 0% casein fed nymphs was extended 2 weeks.

First instar nymphs were fed four A9248 bait concentrations (2, 4, 8 and 12%) prepared in either a casein

or laboratory rodent chow substrate to determine an effective dosage. Figures 5.2 and 5.3 compare the results of these bait studies. With the exception of the 2% casein bait, all other baits, regardless of dose and substrate, were equally effective in causing 100% mortality in treated populations. However, the percent survival on 4, 8 and 12% casein baits decreased significantly between 2 and 3 weeks posttreatment as indicated by the slopes of the linear regression equations. Percent survival of those cockroaches fed laboratory rodent chow baits at the same concentrations decreased significantly between 1 and 2 weeks. Both 2% baits exhibited a 1-2 week delay in their activity. Only the population fed 2% casein bait had an increase in total body mass 2.5x (Kramer, unpublished data). The 20% casein control population had experienced 68% mortality by the time hatch occurred (11 weeks), while lab chow controls experienced only 9% mortality at first hatch (9 weeks). The mean body mass of the population fed the untreated 20% casein bait increased 24.7x (9 weeks) compared to the 27.3x (7 weeks) increase in the total body mass of the population fed untreated lab chow bait.

Topical application of a 4% (1ul/insect) A9248 acetone solution was used to assess the toxicity of this compound when externally applied. At 30 days postexposure 29 of 30 treated males had survived and an identical number of acetone treated males were alive, indicating a lack of

Figure 5.2. Percent survival of 1st instar German cockroach nymphs fed various concentrations of A9248 casein baits. Results of linear regression analyses (slope ± SEM; r^2): ■ control (-6.57 ± 0.59; 0.92); + 2% A9248 (-14.53 ± 1.45; 0.93); ◇ 4% A9248 (-28.80 ± 4.63; 0.93); △ 8% A9248 (-23.09 ± 3.80; 0.90); x 12% A9248 (-28.80 ± 4.46; 0.93).

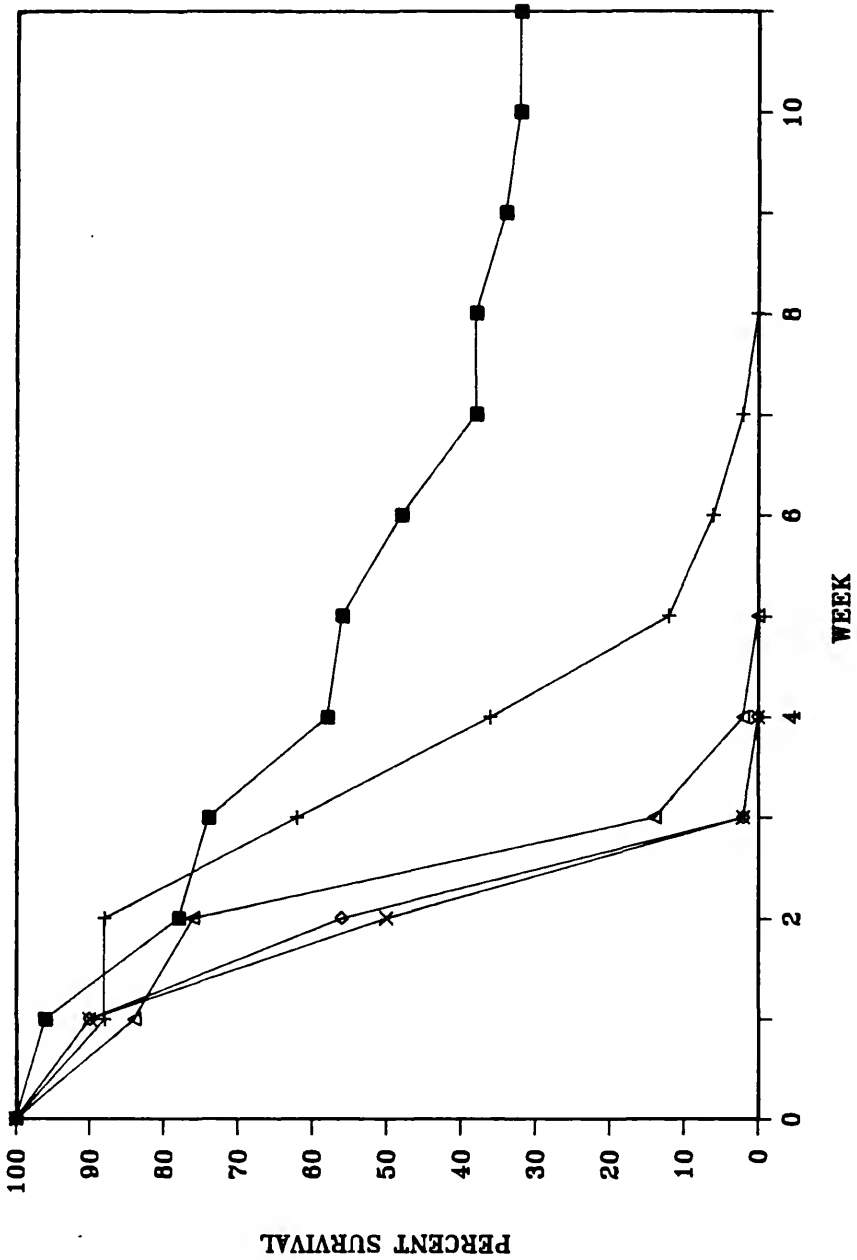
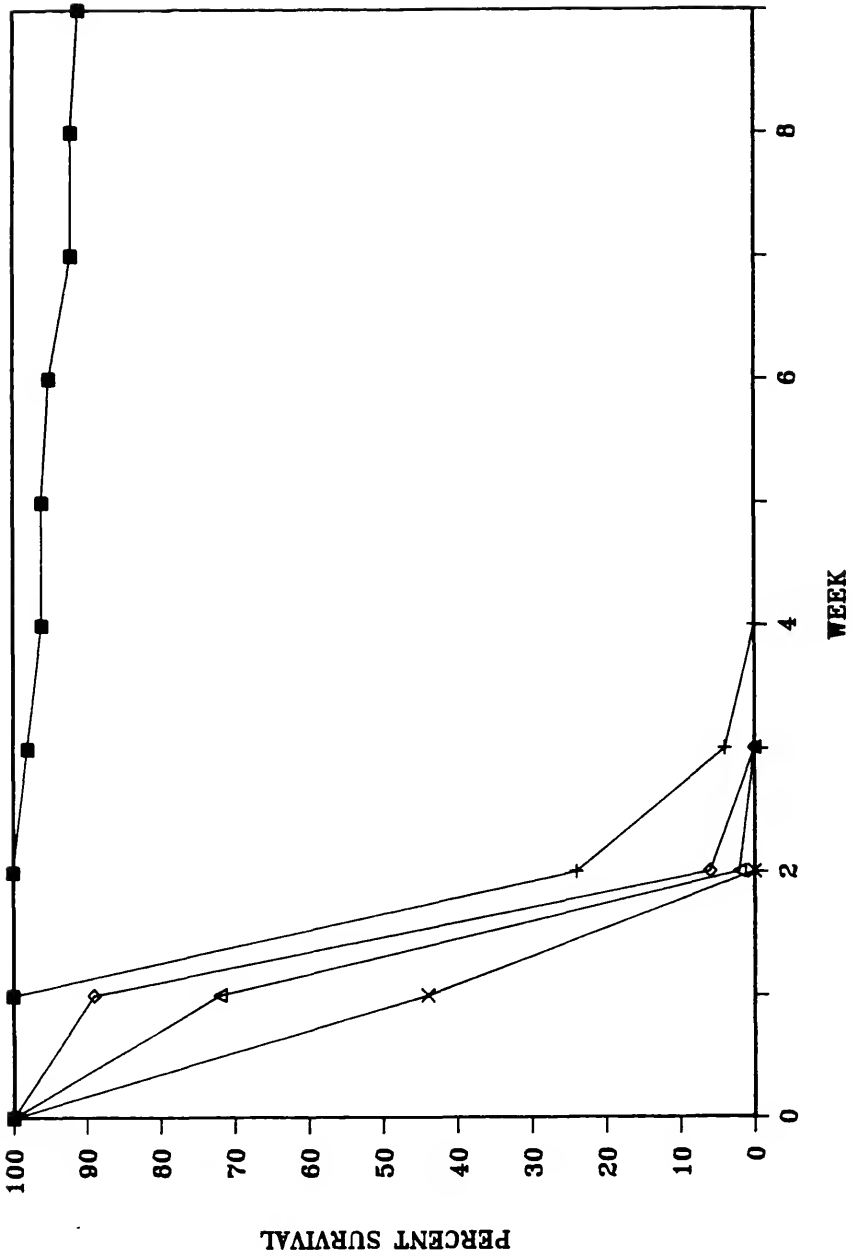

PERCENT SURVIVAL
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
WEEK

Figure 5.3. Percent survival of 1st instar German cockroach nymphs fed various concentrations of A9248 laboratory rodent chow baits. Results of linear regression analyses (slope ± SEM; r^2): ■ control (- 1.13 ± 0.14; 0.79); + 2% A9248 (-29.6 ± 6.91; 0.86); ◇ 4% A9248 (-38.3 ± 10.57; 0.87); △ 8% A9248 (-37.0 ± 8.80; 0.90); x 12% A9248 (-50.0 ± 3.46, 0.99).


PERCENT SURVIVAL
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
WEEK

penetration or topical toxicity. Likewise, residual treatments of 1, 2 and 4% A9248 on plywood did not cause any significant differences in percent survival of males when compared to unexposed controls percent survival for all four treatments ranged from 45-54% after 9 weeks of continuous exposure).

The results of the A9248 4% laboratory rodent chow choice test for 2nd instar nymphs are shown in Figure 5.4. This figure compares the effects of untreated laboratory rodent chow, 4% A9248 laboratory rodent chow bait and a choice of both. Providing a choice of baits moderated the effect of A9248. Percent survival of the population provided a choice was reduced to 32%; that of the control population remained high at 82%; the A9248 laboratory rodent chow bait produced total mortality within 3 weeks. Although delayed until 12 weeks postexposure, reproduction occurred when a choice was offered. A finding substantially different from non-choice tests in that reproduction had not occurred previously because all treated nymphs fed A9248 (nonchoice) expired prior to reaching adulthood.

The combined treatment of 1% A9248 laboratory rodent chow bait (nonchoice) and 0.1% hydroprene residual caused no significant differences in percent survival or reproduction other than those expected under independent conditions (Figure 5.5). Percent survival in the populations fed A9248 and A9248/hydroprene declined to 0% over a 10 week period a

Figure 5.4. Percent survival of 2nd instar German cockroach nymphs: 4% A9248 laboratory rodent chow choice test. Results of regression analyses (slope ± SEM; r^2): ■ control (-1.83 ± 0.26; 0.72); + 4% A9248 only (-45.75 ± 8.76; 0.87); ◊ choice (-6.21 ± 0.21; 0.97).

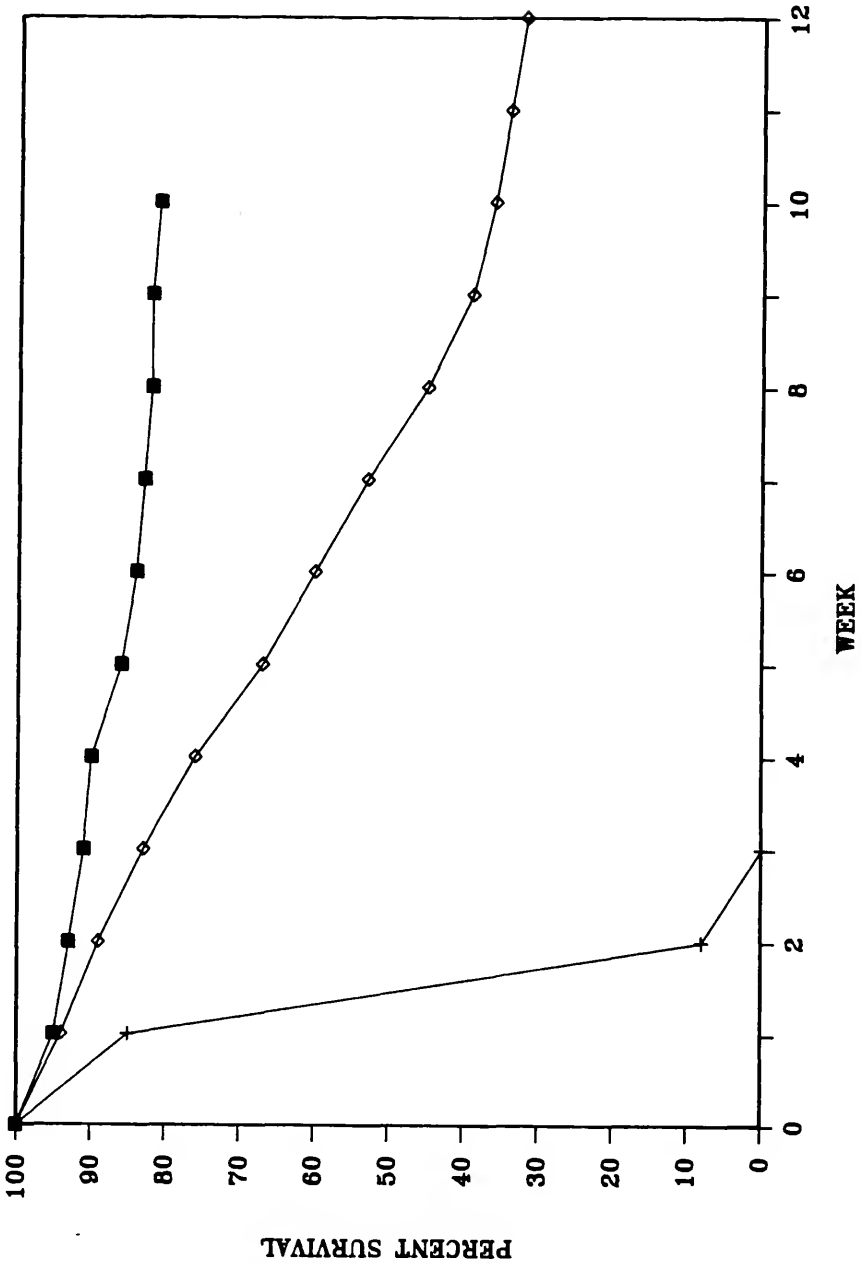
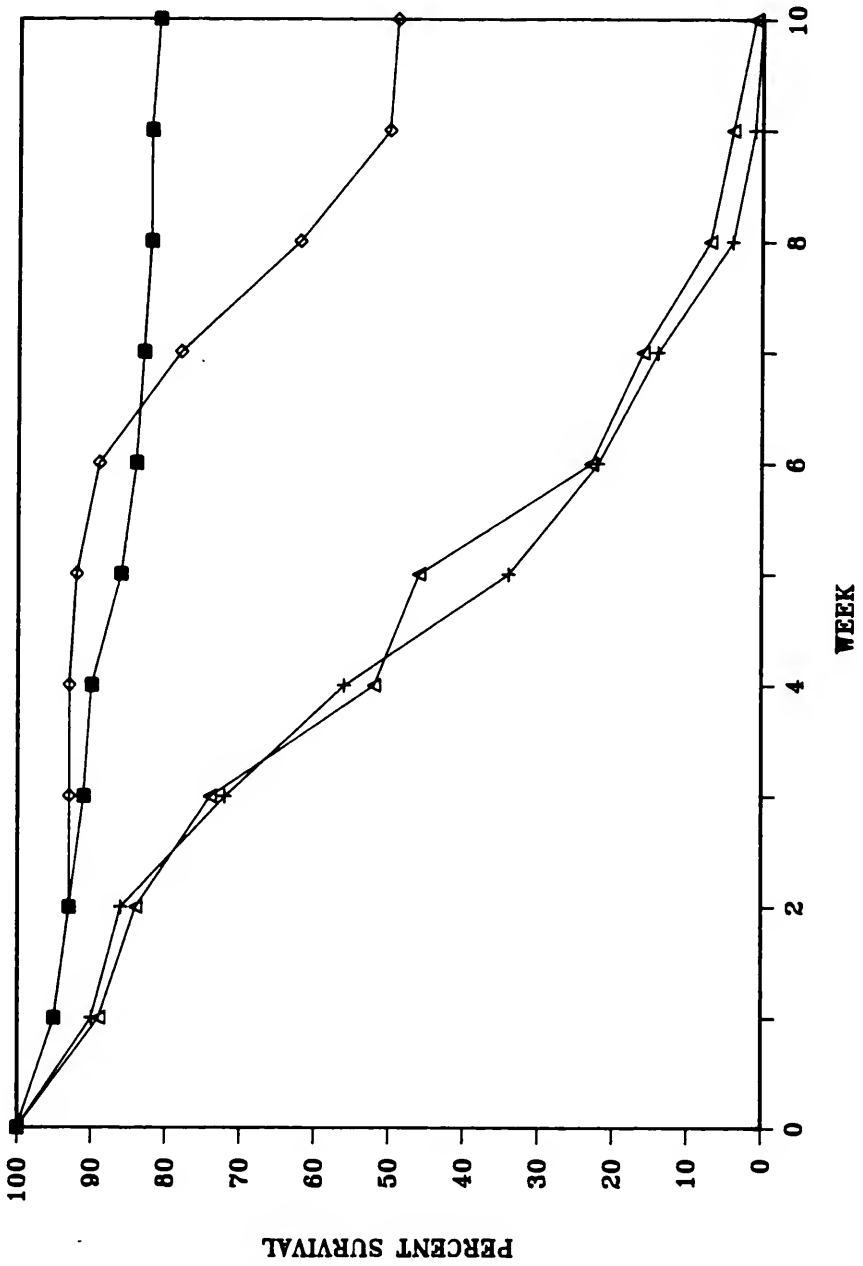

PERCENT SURVIVAL
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
WEEK

Figure 5.5. Percent survival of 2nd instar German cockroach nymphs fed a 4% A9248 laboratory rodent chow bait and exposed to 0.1% hydroprene. Results of linear regression analyses (slope ± SEM; r^2): ■ control (-1.83 ± 0.26; 0.72); + 4% A9248 only (-12.25 ± 0.48; 0.97); ◊ 0.1% hydroprene only (-5.11 ± 0.56; 0.80); △ 4% A9248/0.1% hydroprene (-11.08 ± 0.48; 0.96).

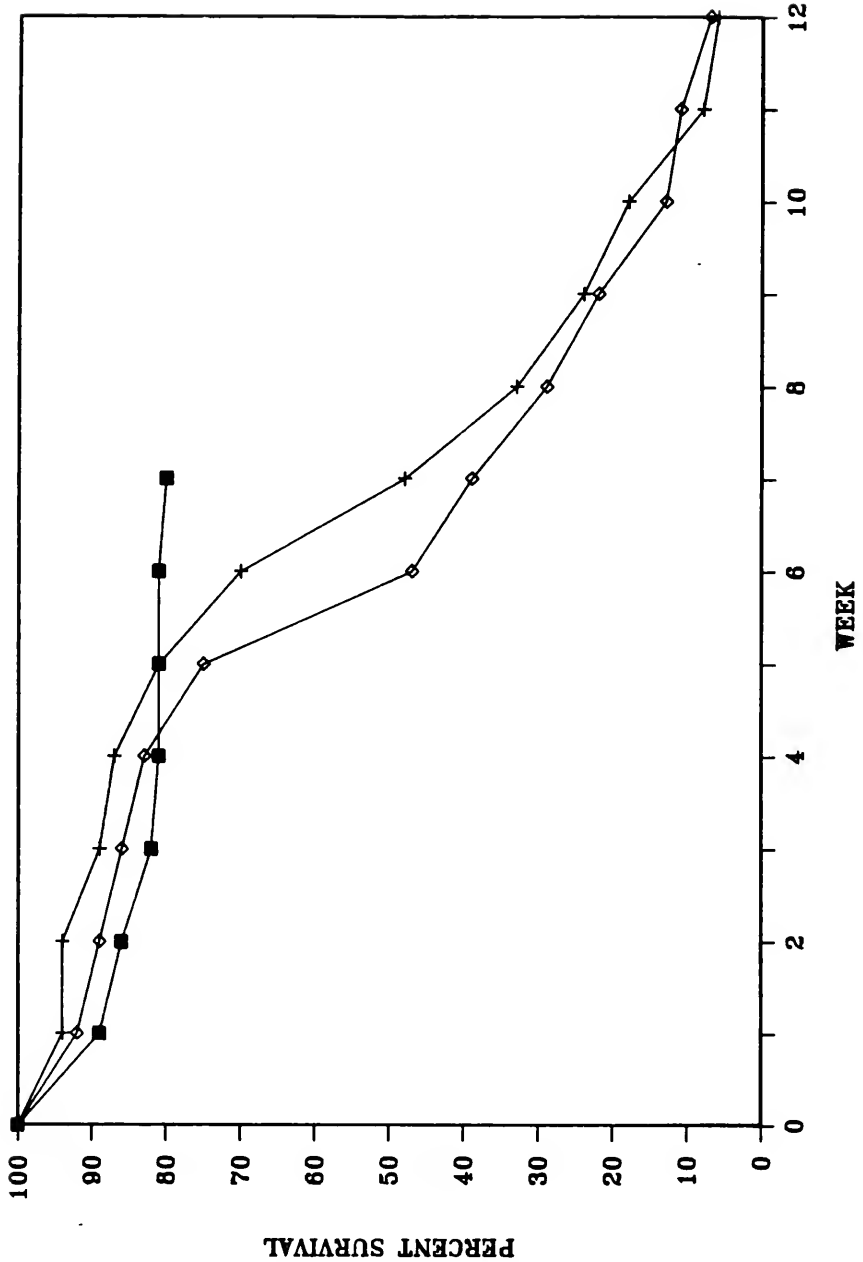

PERCENT SURVIVAL
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
WEEK

much slower rate than in previous studies, presumably due to the lower (1.0%) concentration. The reduction in percent survival of the hydroprene population to 49% compared to 81% for the unexposed population was unexpected (Chapter IV) and can not be explained.

The effects of 2% allopurinol formulated in a 20% casein bait are shown in Figure 5.1 and have been discussed previously. The results of the 2% allopurinol laboratory rodent chow bait studies are represented in Figure 5.6 and include the results of the 0.1% hydroprene residual combined treatment. In comparing the results of allopurinol as a casein bait (Figure 5.1) and as a laboratory rodent chow bait (Figure 5.6), there was no significant difference in the percent survival during the studies, with the exception of a 1 week delay in the onset of survival decline in the latter population. The combination of bait and a 0.1% hydroprene residual treatment apparently did not affect the efficacy of either compound as seen in Figure 5.6. Results of the allopurinol choice test are depicted in Figure 5.7; there was no appreciable difference in percent survival (2% at 10 weeks) of the population given a food choice and the percent survival (6% at 10 weeks) of those provided the no choice casein diet Figure 5.1.

Feeding nymphs 2% allopurinol baits resulted in reproductive failure; all males died within 1-21 days postemergence (most within the first seven days). Emergent

Figure 5.6. Percent survival of 3rd instar German cockroach nymphs fed 2% allopurinol laboratory rodent chow bait and exposed to 0.1% hydroprene. Results of linear regression analyses (slope ± SEM; r^2): ■ control (-2.33 ± 0.62; 0.71); + 2% allopurinol/0.1% hydroprene (-8.97 ± 0.65; 0.95); ◇ 2% allopurinol only (-8.84 ± 0.40; 0.95).


PERCENT SURVIVAL
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
WEEK

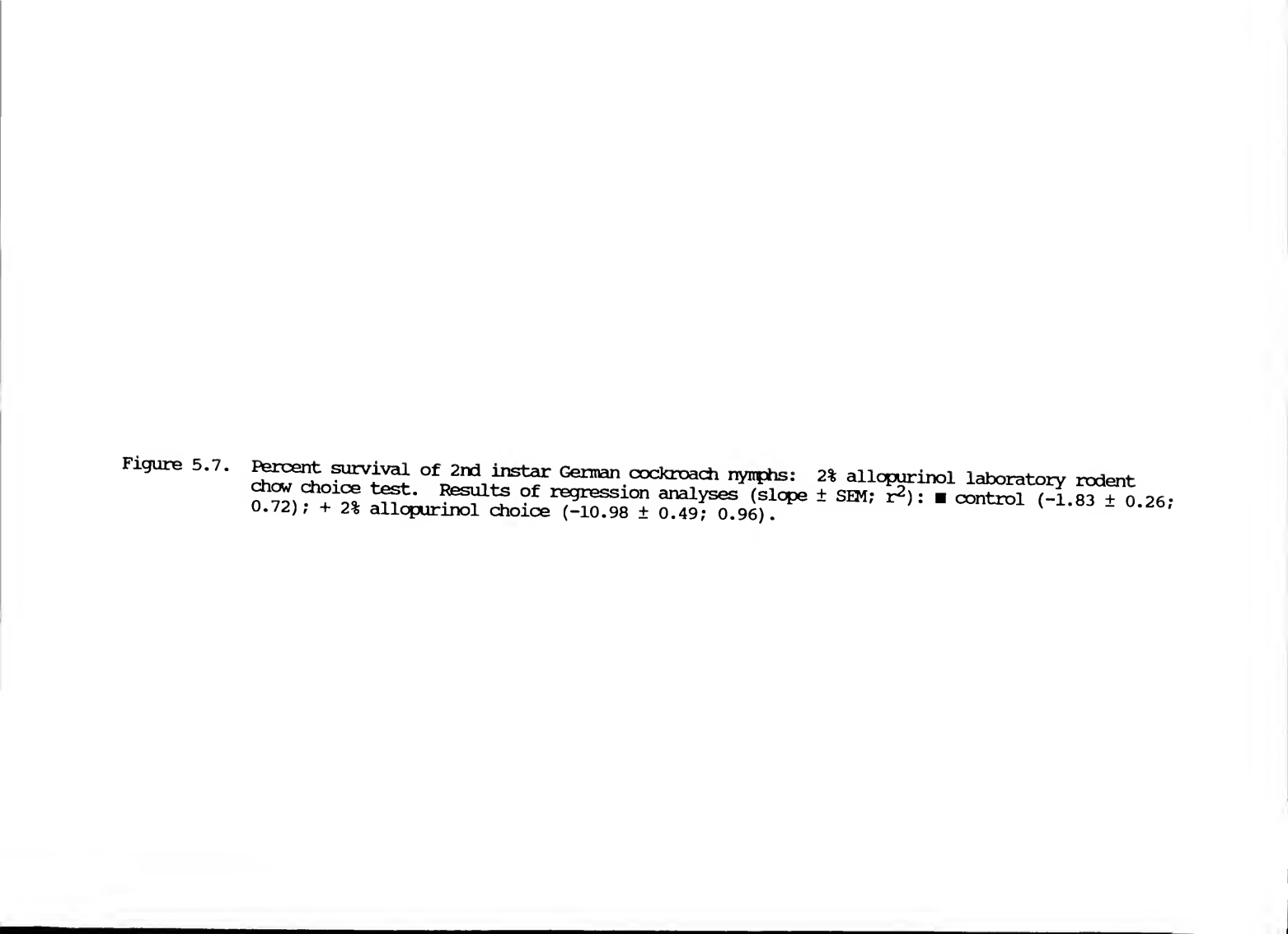
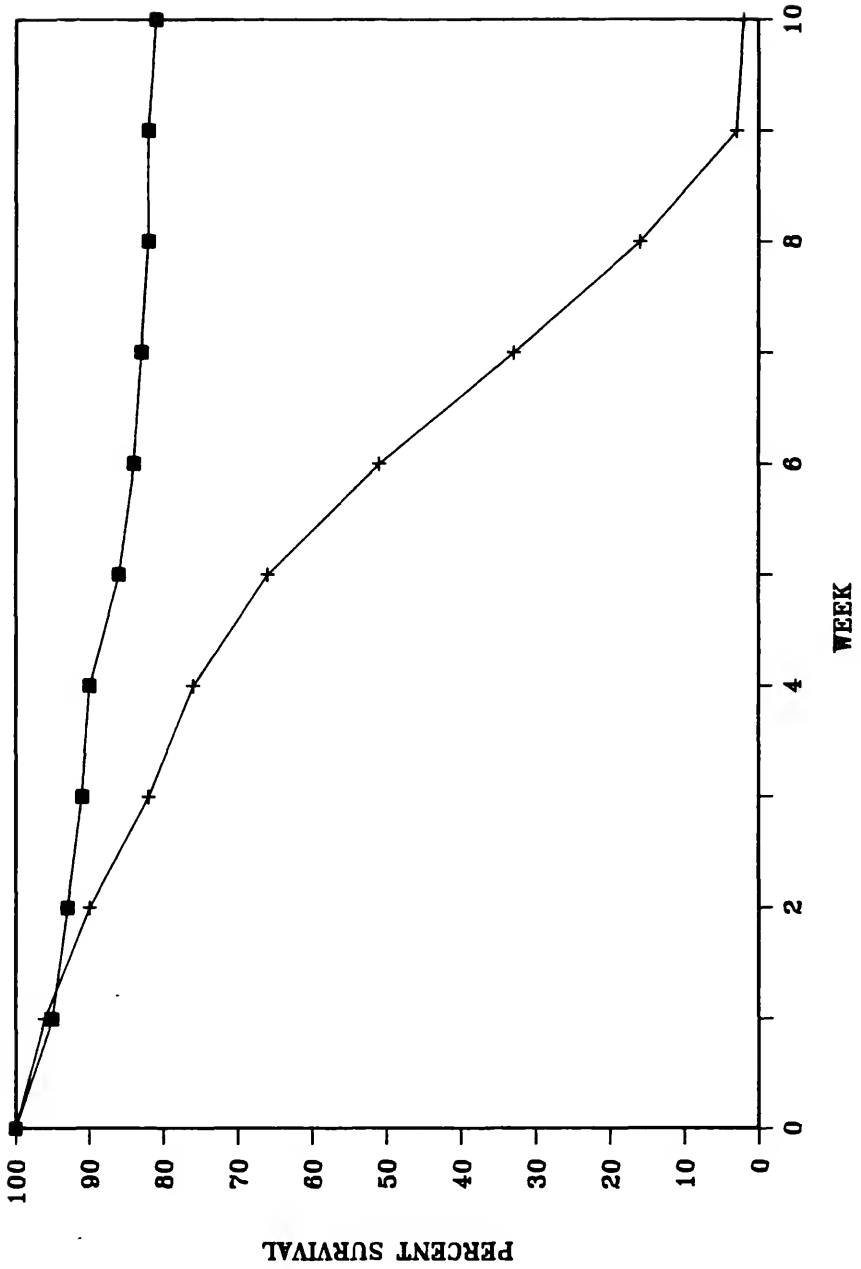


Figure 5.7. Percent survival of 2nd instar German cockroach nymphs: 2% allopurinol laboratory rodent chow choice test. Results of regression analyses (slope ± SEM; r^2): ■ control (-1.83 ± 0.26; 0.72); + 2% allopurinol choice (-10.98 ± 0.49; 0.96).


PERCENT SURVIVAL
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
WEEK

females were observed for a 9-week period and although many produced "false" ootheca, they appeared shriveled or darkened on the protruding end, and all were either dropped or failed to hatch.

Feeding postemergent unmated adults 2% allopurinol bait for 3 days or continuously during the reproductive cycle resulted in a reduction in the number of nymphs/female. Postemergent adult exposure to allopurinol produced the following results: 3 day feeding - 4 nymphs/female, continuous feeding - 1 nymph/female and control - 24 nymphs/female. Reproduction occurred in two-week old mated adults provided a choice of baits; however, no attempt was made to determine the number of nymphs/female.

To determine the effect of allopurinol on total body urates, uric acid analyses were conducted at various developmental times; the results are found in Table 5.1. It is apparent by examining both this table and the data presented in Chapters II and IV, that uric acid accumulates with age in normal German cockroaches. Nymphs fed allopurinol bait fail to accumulate appreciable amounts of uric acid. Males (1-4 days old) fed allopurinol as nymphs had only 8.87 ug/mg dry weight, whereas their normal counterparts, at 1-7 days of age had 88.4 ug/mg dry weight. Females (30 days old) had an analogous pattern (exposed females had 9.79 ug/mg dry weight and normal females had 123.9 ug/mg dry weight). Feeding allopurinol to 14-day old

Table 5.1. Comparison of the mean uric acid content of German cockroaches fed alluprinol bait as nymphs and various stages of unexposed specimens.

	Mean Uric Acid Content (ug/g dry wt)	
Sex	Males	Females
Treatment		
Unexposed		
Penultimate nymphs	66.10c	91.80c
1-day old adults	106.30b	83.10c
1-7 day old adults	88.40bc	91.00c
30-day old adults	161.10a	123.90b
Allopurinol Treated		
1-4 day old adults	8.87d	-
30-day old adults	-	9.79d
30-day old adults fed bait 14 days postemergence	161.30a	133.90a

Means within a column followed by the same letter were not significantly different (P = 0.05; Waller-Duncan Method [SAS Institute, 1985]).

postemergent adults did not deplete the amount of total body urates when compared to unexposed adults of similar age (30 days old).

Discussion

German cockroaches are opportunistic feeders and can grow and reproduce on a wide range of nutritional resources. The effects of varying the amount of dietary protein on survival, metabolic reserves and insecticide tolerance in German cockroaches have been discussed in Chapter II and by Haydak (1953), Melampy and Maynard (1937) and Willis (1958). Widespread resistance in this species necessitates the development of novel pest management strategies that are effective and, yet, will not cause environmental insult. The two compounds evaluated in this study showed promise of fulfilling both these criteria.

The antibiotic properties of A9248 and its effectiveness in destroying symbiotic microorganisms, as demonstrated by Breznak (unpublished data), was probably the principal factor involved in reducing the percent survival of exposed populations. German cockroaches have been rendered aposymbiotic with chlortetracycline HCl treatment and, reportedly, die when fed a dog chow diet, or they experience a protracted development period with little growth when fed a casein diet (Brooks and Kringen, 1972).

Restricting the diet of German cockroach nymphs to A9248 casein baits produced similar results (lack of growth and 100% mortality in 3-4 weeks). The laboratory rodent chow baits reduced the percent survival more precipitously (ca. 2 weeks), probably as a result of higher acceptability and/or increased consumption rate due to less utilizable protein. Repellency did not appear to be a factor because higher dosages produced identical results. Cockroaches ingesting these baits excreted large droplets of a brown exudate rather than dry fecal pellets indicating that A9248 may interfere with normal digestion and absorption. Only at the 2% concentration was total mortality delayed. Based on the results of this study, a 4% concentration of A9248 was most effective against all nymphal stages in terms of efficacy and economy.

Although A9248 baits resulted in 100% mortality in no choice situations, their efficacy was mitigated when a choice was available (percent survival was 32% and reproduction occurred). Similar results were obtained when baits were used in conjunction with hydroprene residual treatments; however, reproduction did not occur, as would be expected with hydroprene exposure (Staal, 1985). Although this compound reduces German cockroach populations, it would not be expected to provide adequate control under field conditions.

A9248 warrants further investigation. It would be beneficial to know whether the mycetocyte bacteria are destroyed when exposed to A9248 and to know the composition of the liquid exudate which might provide information on how A9248 affects the digestive process.

While A9248 presumably attacks the mycetocyte component of the fat body complex, allopurinol was shown to affect a second component, the urocytes, and their metabolic reserve, uric acid. The reduction of total body urates in allopurinol-fed adults demonstrated by Engebretson and Mullins (1986) differred from the results of this study, no significant reduction in a similar treatment group. The importance of this fat body complex and the uric acid reserve has been documented and reviewed by Cochran (1985) and appears to play a major role in the survival of many cockroach species. Uric acid functions as a nitrogen and carbon metabolic reserve in B. germanica (Cochran, 1985), as a paternal investment during copulation and as a subsequent component of the ootheca (Mullins and Keil, 1980), and it may well serve as a cation sink (Tucker, 1977a). The production and storage of uric acid has the added benefit of minimizing water loss associated with the other means of nitrogenous waste excretion.

Allopurinol's ability to inhibit the deposition of uric acid would be expected to disrupt homeostasis and affect reproduction, as occurred in this study. Nymphs fed a 2%

allopurinol laboratory rodent chow bait in either a choice or nonchoice situation experienced ca. 1 week delay in adult emergence, reduced survival (2-7%) at 10-12 weeks and reproductive failure. Combining the bait treatment with a 0.1% hydroprene residual caused no significant change in these findings. Postemergent adults fed the 2% bait experienced a significant reduction in the number of nymphs produced per exposed female.

The reduction (ca. 10x) in whole body urates, the reduced longevity of emergent adults, particularly males (1-21 days), and the absence of reproduction seem to underscore the importance of this metabolic reserve. It is feasible that the inability of males and/or females to produce sufficient amounts of uric acid accounts for the reproductive failure noted. The shortened longevity of males may be a factor, although this is unlikely because males may exhibit copulatory behavior at 1-2 days postemergence (Roth and Willis, 1952). The significant reduction in percent survival of populations fed allopurinol bait when compared to control populations indicates that uric acid formation and storage may play a more important role in cockroaches than previously thought and warrants further investigation. The metabolic activity of the fat body complex, trophocytes, urocytes and mycetocytes, may be so well balanced that the depletion of just one unit produces these deleterious results. Evidence for this

hypothesis was previously presented in the discussion regarding possible effects of A9248 on the mycetocyte bacteria.

Allopurinol 2% laboratory rodent chow baits can be used effectively to eliminate German cockroach populations under laboratory conditions. This compound offers a novel approach--inhibition of uric acid formation--in controlling this species. However, further testing is needed to confirm its effectiveness in arena tests and subsequently under field conditions. Totally different control rationales must be fostered, as suggested by Reierson (1986), if we expect to succeed in controlling this pest.

CHAPTER VI
SUMMARY AND RECOMMENDATIONS

Results of this study are summarized as follows:

1. Water and/or food deprivation for 3 days significantly increased susceptibility of male German cockroaches to propoxur but caused little change in susceptibility of cockroaches treated with chlorpyrifos.

2. A multiresistant HRDC strain was more susceptible to propoxur after 5 days starvation than was the Orlando normal strain. Susceptibility of the HRDC strain to chlorpyrifos was reduced, but resistance ratios remained elevated at 6:4 and 11:2 for each dosage level.

3. Amount of dietary protein had no effect on susceptibility of male German cockroaches to propoxur or to chlorpyrifos. However, the source of protein produced significant differences in their tolerance of these insecticides. Males fed a laboratory rodent chow diet were 2x more tolerant than those fed a casein diet.

4. Total body mass, carbohydrates, lipids and uric acid were related to the type of diet consumed. In males fed a laboratory rodent chow diet, total body lipids, carbohydrates and uric acid were significantly higher than in those fed a casein diet and may account for increased tolerance.

5. The casein diet did not promote optimal growth of German cockroaches, in contrast to the laboratory rodent chow diet.

6. Colonization for extended time periods on a wide range of dietary protein failed to produce genetic drift, as determined by starch gel electrophoresis.

7. Within 2 hours of receiving a sublethal dose of propoxur, male German cockroaches lost significant amounts of water (ca. 10% of their total body mass). Similar losses occurred at 24 hours postexposure, although water loss in controls also increased.

8. Water consumption, after sublethal exposure, was elevated in both the treated and control groups, but they were not significantly different. Male German cockroaches consumed water (2.30-12.45 ul) daily; however, the percent of treated males consuming water on days 3-4 posttreatment was lower than in untreated controls. Food consumption in both groups was highest on the first day posttreatment, but there was no significant difference in the amount consumed. Most males consumed food at 2 day intervals.

9. The IGR, hydroprene, induced morphogenetic changes in the left and right phallomeres, subgenital plate and styli and were related to dose and age of nymphal exposure. The malformation of the left phallomere and noneversibility of the right phallomere precluded copulation.

10. Hydroprene exposure increased emergent adult body mass (at 30 days) by as much as 24% in males and 33% in

females. At 12 weeks postemergence, the body mass of treated males was as much as 53% greater than of untreated males, and the body mass of treated females was as much as 47% greater than that of untreated females. Percent body water of males varied little; however, mean dry weight was much higher in treated males. Percent body water of treated females was as much as 11% less than that of untreated females, and the mean dry weights were significantly higher.

11. Hydroprene concentration resulted in varied effects on the total body mass of males and females. Generally, at 60 days postemergence, wet and dry weights increased with dose and percent body water decreased.

12. The changes in total body mass were reflected as changes in total body carbohydrates, lipids and urates. Hydroprene treated females had significantly higher posttreatment carbohydrate levels which decreased with age; deposition of lipids were high initially but declined with age; total body urates increased throughout the 90 day period but plateaued between 30 and 60 days. Hydroprene-treated males exhibited increasing levels of total body carbohydrates with age; total body lipids declined with age and plateaued between 60 and 90 days; total body urates increased significantly throughout the postemergent period.

13. Hydroprene disrupts the homeostatic balance in German cockroaches; however, only slight differences in treated male susceptibility to propoxur or to chlorpyrifos were shown when compared with untreated males. Orlando

normal and HRDC males exposed to a lower hydroprene dose were more susceptible to propoxur than were unexposed males. Orlando normal males treated with hydroprene exhibited a slight increase in tolerance to chlorpyrifos. Hydroprene-treated HRDC males were more susceptible to chlorpyrifos when exposed to a low dose of hydroprene and were more tolerant when exposed to a higher dose.

14. A9248 and allopurinol were evaluated as growth inhibitors for German cockroaches. A9248 formulated as a 4% bait appeared to affect the digestive process and, in nonchoice tests, caused 100% mortality in treated nymphs. In choice tests, however, percent survival was ca. 32%, and reproduction occurred.

15. Allopurinol fed as a 2% bait inhibited the accumulation of uric aid in German cockroaches, reduced percent survival to between 2 and 7% and survivors failed to reproduce. Allopurinol bait was equally effective in choice as nonchoice bait tests. Adults fed the baits during the postemergent period suffered significant reductions in the number of nymphs per female.

16. Neither A9248 nor allopurinol enhanced the activity of hydroprene when used as a combined treatment.

Recommendations for future investigation include:

1. Studying the effects of nutritional stresses on the susceptibility of field populations subject to variations in abiotic factors, foraging costs, assimilation efficiencies and consumption rates.

2. Determining the cause of the lack of propoxur resistance in the multiresistant HRDC strain in the face of continuous pressuring with this insecticide.

3. Determining the specific cause for excessive water loss during propoxur intoxication.

4. Developing improved techniques for determining food consumption rates.

REFERENCES

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Appaiah, K. M., A. Rajanna, and B. V. V. Rao. 1973. Effect of protein and carbohydrate nutrition on the resistance of American cockroaches (Periplaneta americana L.) to malathion. Current Research 2: 11.

Appel, A. G., D. A. Reierson, and M. K. Rust. 1983. Comparative water relations and temperature sensitivity of cockroaches. Comp. Biochem. Physiol. 74A: 357-361.

Barton-Browne, L. 1975. Regulatory mechanisms in insect feeding. Adv. Insect Physiol. 11: 1-116.

Beck, S. D., and J. F. Stauffer. 1957. The European corn borer, Pyrausta nubilalis (Hubn.) and its principal host plant III toxic factors influencing larval establishment. Ann. Entomol. Soc. Am. 50: 166-170.

Beenakkers, A. M. T. 1983. Regulation of lipid metabolism, pp. 441-450. In R. G. H. Downer and H. Laufer (eds.), Endocrinology of insects. Alan R. Liss, Inc., New York.

Bennett, G. W., J. W. Yonker, and E. S. Runstrom. 1986. Influence of hydroprene on German cockroach (Dictyoptera: Blattellidae) populations in public housing. J. Econ. Entomol. 79: 1032-1035.

Bilbie, I., and G. Nicolescu. 1981. The simultaneous action of juvenile hormone analogue, 1-(4'-ethylphenoxy)-6,7-epoxy-3, 7-dimethyl-2-octene and organophosphorus insecticide trichlorfon in Blattella germanica L. species. Arch. Roum. Path. Exp. Microbiol. 40: 173-175.

Brenner, R. J., P. G. Koehler, and R. S. Patterson. Integration of the IGR fenoxycarb in a German cockroach management program. (Dictyoptera: Blattellidae). J. Econ. Entomol. (in press).

Brett, B. L., and M. H. Ross. 1985. Insecticide-induced dispersal in the German cockroach, Blattella germanica (L.) (Orthoptera: Blattellidae). J. Econ. Entomol. 78: 1293-1298.

Brooks, M., and W. B. Kringen. 1972. Polypeptides and proteins as growth factors for aposymbiotic Blattella germanica (L.), pp. 353-364. In J. G. Rodriquez (ed.),Insect and mite nutrition. North Holland, Amsterdam.

Cochran, D. G. 1982. Cockroaches--biology and control. World Health Organization.

Cochran, D. G. 1983. Food and water consumption during the reproductive cycle of female German cockroaches. Ent. Exp. Appl. 34: 51-57.

Cochran, D. G. 1985. Nitrogen excretion in cockroaches. Ann. Rev. Entomol. 30: 29-49.

Collins, W. J. 1973. German cockroach resistance 1. Resistance to diazinon includes cross-resistance to DDT, pyrethrins, and propoxur in a laboratory colony. J. Econ. Entomol. 66: 44-47.

Cornwell, P. B. 1968. The cockroach, vol. I. A laboratory insect and industrial pest. Hutchinson and Co., London.

Cornwell, P. B. 1976. The cockroach, vol. II. Insecticides and cockroach control. Associated Business Programmes, London.

Cristodorescu, G., I. Nosec, and V. Tacu. 1978. The action of juvenile hormone analogue, 1-(4'-ethylphenoxy)-6,7-epoxy-3,7-dimethyl-2-octene, in Blattella germanica by continuous treatment (note III.). Arch. Roum. Path. Exp. Microbiol. 37: 47-53.

Dadd, R. H. 1970. Arthropod nutrition. Chap. 2 in Chemical zoology, vol. 5. Academic Press, Inc., New York.

Das, Y. T., and A. P. Gupta. 1974. Effects of three juvenile hormone analogs on the female German cockroach, Blattella germanica (L.) (Dictyoptera: Blattellidae). Experientia 30: 1093-1095.

Das, Y. T., and A. P. Gupta. 1977. Abnormalities in the development and reproduction of Blattella germanica (L.) (Dictyoptera: Blattellidae) treated with insect growth regulators with juvenile hormone activity. Experientia 33: 968-970.

Edwards, A. J. 1981. Effects of carbon dioxide anesthesia and crowding on the susceptibility of cockroaches to insecticides. Ent. Exp. Appl. 29: 339-344.

Engebretson, J. A., and D. E. Mullins. 1983. The effects of dietary nitrogen levels on glycine, formate, and xanthine incorporation into the urates in the German cockroach, Blattella germanica L. (Dictyoptera: Blattellidae). Comp. Biochem. Physiol. 75B: 293-300.

Engebretson, J. A., and D. E. Mullins. 1986. Effects of a purine inhibitor, allopurinol, on urate metabolism in the German cockroach, Blattella germanica L. (Dictyoptera:Blattellidae). Comp. Biochem. Physiol. 83B: 93-97.

Frishman, A. 1982. Cockroaches. In A. Mallis (ed.), Handbook of pest control, 6th ed. Franzak and Foster, Cleveland, Ohio.

Gordon, H. T. 1959. Minimal nutritional requirements of the German cockroach, Blattella germanica L. Annals N.Y. Acad. Sci. 77: 290-351.

Gordon, H. T. 1961. Nutritional factors in insect resistance to chemicals. Ann. Rev. Entomol. 6: 27-54.

Gordon, H. T. 1968. Intake of various solid carbohydrates by male German cockroaches. J. Insect Physiol. 14: 41-52.

Gordon, H. T. 1972. Interpretations of insect quantitative nutrition, pp. 73-105. In J. G. Rodriquez (ed.), Insect and mite nutrition. North Holland, Amsterdam.

Grayson, J. M. 1951. Response of the German cockroach to sublethal concentrations of DDT and benzene hexachloride. J. Econ. Entomol. 44: 315-317.

Hampson, B. C., and W. W. M. Steiner. 1982. An electromorphetic analysis of population structure and gene diversity in the German cockroach, pp. 648-663. In W. W. M. Steiner, W. J. Tabachnik, L. S. Rai, and S. Narang (eds.), Recent developments in the genetics of insect disease vectors. Stipes Publishing Co., Champagne, Illinois.

Hangartner, W., and P. Masner. 1973. Juvenile hormone: inhibition of ecdysis in larvae of the German cockroach, Blattella germanica. Experientia 29: 1358-1359.

Harris,H., and D. Hopkinson. 1976. Handbook of enzyme electrophoresis in human genetics. Elsevier North-Holland, New York.

Haydak, M. H. 1953. Influence of the protein level of the diet on the longevity of cockroaches. Ann. Entomol. Soc. Am. 6: 547-560.

House, H. L. 1961. Insect nutrition. Ann. Rev. Entomol. 6: 13-26.

Koehler, P. G., H. R. Agee, N. C. Leppla, and R. S. Patterson. 1987. Spectral sensitivity and behavioral response to light quality in the German cockroach (Dictyoptera: Blattellidae). Ann. Entomol. Soc. Am. (in press).

Lanzorein, B. 1974. Influence of a juvenile hormone analogue on vitellogenin synthesis and oogenesis in larvae of Nauphoeta cinerea. J. Insect Physiol. 20: 1871-1885.

Lehninger, A. L. 1982. Principles of biochemistry. Worth Publishers, Inc. New York.

Lockwood, J. A., T. C. Sparks, and R. N. Story. 1984. Evolution of insect resistance to insecticides: a reevaluation of the roles of physiology and behavior. Bull. Entomol. Soc. Amer. 41-51.

Lofgren, C. S., and L. K. Cutcomp. 1956. Toxicity of DDT to the American cockroach when lipid content and temperature are varied. J. Econ. Entomol. 49: 167-171.

Mansingh, A. 1965. Cholinesterase activity of susceptible and resistant strains of malathion-poisoned German cockroaches. J. Econ. Entomol. 58: 580-581.

Mansour, M. H. 1978. Inhibitory and stimulatory effects of low doses of insecticides on growth and reproductivity of the cotton leafworm Spodoptera littoralis Boisd. Zeitschrift fur Pflanzenkrankheifen Pflanzenschutz 85: 570-575.

Masner, P., and W. Hangartner. 1973. Ecdysone: an antagonist of juvenile hormone in the control of cuticle synthesis in the German cockroach (Blattella germanica). Experientia 29: 1550-1551.

Masner, P., W. Hangartner, and M. Suchy. 1975. Reduced titers of ecdysone following juvenile hormone treatment in the German cockroach, Blattella germanica. J. Insect Physiol. 21: 1755-1762.

Matsumura, F. 1975. Toxicology of insecticides. Plenum Press, New York.

Melampy, R. M., and L. A. Maynard. 1937. Nutritional studies with the cockroach, Blattella germanica. Physiol. Zool. 10: 36-44.

Milio, J. F., P. G. Koehler, and R. S. Patterson. 1987. Laboratory and field evaluations of hydramethylon bait formulations for control of American and German cockroaches (Orthoptera: Blattellidae). J. Econ. Entomol. 79: 1280-1286.

Mullins, D. E., and D. G. Cochran. 1975. Nitrogen metabolism in the American cockroach - I. An examination of positive nitrogen balance with respect to uric acid stores. Comp. Biochem. Physiol. 50A: 489-500.

Mullins, D. E., and D. G. Cochran. 1983. Nitrogen metabolism, pp. 451-466. In R. G. H. Downer and H. Laufer (eds.), Endocrinology of insects. Alan R. Liss, Inc., New York.

Mullins, D. E., and C. B. Keil. 1980. Paternal investment of urates in cockroaches. Nature 283: 567-569.

Nation, J. L. 1983. A new method for using hexamethyldisilazane for preparation of soft insect tissues for scanning electron microscopy. Stain Technology 58: 347-351.

Noland, J. L., and C. A. Baumann. 1951. Protein requirements of the cockroach Blattella germanica (L.). Ann. Entomol. Soc. Am. 44: 184-189.

Noland, J. L., J. H. Lilly, and C. A. Baumann. 1949. A laboratory method for rearing cockroaches, and its application to dietary studies on the German roach. Ann. Entomol. Soc. Am. 42: 63-67.

Nosec, I., A. Cristescu, A. Enescu, V. Tacu, I. Giurca, G. Cristodorescu, and S. Durbaca. 1977. The estimation of the effect of some hormonal analogs on four insect species of sanitary-medical importance. Arch. Roum. Path. Exp. Microbiol. 36: 61-65.

Owens. J. M., and G. W. Bennett. 1982. German cockroach movement within and between urban apartments. J. Econ. Entomol. 75: 570-573.

Patterson, R. S, and P. G. Koehler. 1985. Sterility: a practical IPM approach for German cockroach (Blattella germanica) control. Proceedings of the First Insect Growth Regulator Symposium pp. 48-60.

Perry, A. S., and M. Agosin. 1974. The physiology of insecticide resistance by insects, pp. 3-121. In M. Rockstein (ed.), The physiology of insecta, vol. VI. Academic Press, New York.

Pluthero, F. G., and R. S. Singh. 1984. Insect behavioral responses to toxins: practical and evolutionary considerations. Can. Entomol. 116: 57-68.

Reichenbach, N. G., and W. J. Collins. 1984. Multiple logit analysis of the effects of temperature and humidity on the toxicity of propoxur to German cockroaches and western spruce budworm larvae. J. Econ. Entomol. 77: 31-35.

Riviere, J. L. 1977. Action du propoxur sur la reproduction de Blattella germanica (L.). Ann. Zool. Ecol. Anim. 9: 111-116.

Ross, M. H., and D. G. Cochran. 1975. The German cockroach, Blattella germanica. In R. C. King (ed.), Handbook of genetics 3: 35-62.

Roth, L, M., and E. R. Willis. 1952. A study of cockroach behavior. Amer. Midland Naturalist 47: 66-129.

Rust, M. R., and D. A. Reierson. 1977. Using pheromone extract to reduce repellency of blatticides. J. Econ. Entomol. 70: 34-38.

Rust, M. R., and D. A. Reierson. 1978. Comparison of the laboratory and field efficacy of insecticides used for German cockroach control. J. Econ. Entomol. 71: 704-708.

SAS Institute. 1979. SAS probit analysis. SAS Institute, Cary, North Carolina.

SAS Institute. 1985. Waller-Duncan method. SAS Institute, Cary, North Carolina.

Schneider, B. M., and G. W. Bennett. 1985. Comparative studies of several methods for determining the repellency of blatticides. J. Econ. Entomol. 78: 874-878.

Slama, K., and C. M. Williams. 1966. 'Paper factor' as an inhibitor of embryonic development of the European bug, Pyrrhocoris apterus. Proc. Nat. Acad. Sci. USA 54: 411-414.

Slansky, F. 1982. Insect nutrition: an adaptionist's perspective. Fla. Entomol. 65: 45-67.

Sroka, P., R. H. Barth, L. I. Gilbert, and G. B. Staal. 1975. Insect juvenile hormone mimics: a structural basis for gonadotropic activity in a cockroach and a moth. J. Insect Physiol. 21: 463-469.

Staal, G. B. 1975. Insect growth regulators with juvenile hormone activity. Ann. Rev. Entomol. 20: 417-460.

Staal, G. B. 1986. Anti juvenile hormone agents. Ann. Rev. Entomol. 31: 391-429.

Staal, G. B., C. A. Henrick, D. L. Grant, D. W. Moss, M. C. Johnston, R. R. Rudolph, and W. A. Donahue. 1985. Cockroach control with juvenoids, pp. 201-218. Am. Chem. Soc. Symp. Ser. No. 276.

Steele, J. E. 1983. Endocrine control of carbohydrate metabolism in insects, pp. 427-440. In R. G. H. Downer and H. Laufer (eds.), Endocrinology of insects. Alan R. Liss, Inc., New York.

Steiner, W. W. M., and D. J. Josslyn. 1979. Electrophoretic techniques for the genetic study of mosquitoes. Mosq. News 39: 35-54.

Terriere, L. C. 1982. The biochemistry and toxicology of insecticides. Oregon State University, Corvallis, Oregon.

Tobe, S. S., and B. Stay. 1979. Modulation of juvenile hormone synthesis by an analog in the cockroach. Nature 281: 481-482.

Tucker, L. E. 1977a. The influence of diet, age and state of hydration on Na^+ and urate balance in the fat body of the cockroach Periplaneta americana. J. Exp. Biol. 71: 67-79.

Tucker, L. E. 1977b. The influence of age, diet and lipid content on survival, water balance and Na^+ and K^+ regulation in dehydrating cockroaches. J. Exp. Biol. 71: 81-93.

Tucker, L. E. 1977c. Regulation of ions in the hemolymph of the cockroach Periplaneta americana during dehydration and rehydration. J. Exp. Biol. 71: 95-110.

Valovage, W. D., and M. A. Brooks, 1979. Uric acid quantities in the fat body of normal and aposymbiotic German cockroaches, Blattella germanica. Ann. Entomol. Soc. Am. 72: 687-689.

Van Handel, E. 1985. Rapid determination of glycogen and sugars in mosquitoes. J. Am. Mosq. Control Assoc. 1: 299-301.

Vogel, W., P. Masner, O. Graf, and S. Dorn. 1979. Types of response of insects on treatment with juvenile hormone active insect growth regulators. Experientia 35: 1254-1256.

Waldbauer, G. P. 1968. The consumption and utilization of food by insects. Adv. Insect Physiol. 5: 229-288.

Weaver, J. E., J. W. Begley, and V. A. Kondo. 1984. Laboratory evaluation of alsystin against the German cockroach (Orthoptera: Blattellidae): effects on immature states and sterility. J. Econ. Entomol. 77: 313-317.

Williams, J. P., J. R. Sauer, R. W. McNew, and J. A. Hair. 1986. Physiological and biochemical changes in unfed lone star ticks, Amblyomma americanum (Acari: Ixodidae), with increasing age. J. Med. Entomol. 23: 230-235.

Willis. E. R., and N. Lewis. 1957. The longevity of starved cockroaches. J. Econ. Entomol. 50: 438-440.

Willis, E. R., G. R. Riser, and L. M. Roth. 1958. Observations on reproduction and development in cockroaches. Ann. Entomol. Soc. Am. 51: 53-69.

Yu, S. J. 1982. Induction of microsomal oxidases by host plants in the fall armyworm, Spodoptera frugiperda (J. E. Smith). Pestic. Biochem. Physiol. 17: 59-67.

BIOGRAPHICAL SKETCH

Richard D. Kramer was born on 13 September, 1947 in Allentown, Pennsylvania, but has been a resident of Florida since moving here in 1953.

Following graduation from Satellite High School in 1965, Richard entered the University of Florida from which he received the Bachelor of Science in Agriculture degree in 1969. After receiving a Regular Army commission that year, he served for nearly four years as a medical supply officer at the 10th Medical Laboratory in Landstuhl, West Germany.

While still on active duty in the U. S. Army, Richard entered graduate school at the University of Florida in September 1973 to study medical-veterinary entomology and received the Master of Science degree in August 1975.

Since that time, he has served as an entomological survey officer at Fort McPherson in Atlanta, Georgia, as an instructor of entomology and preventive medicine at the Academy of Health Sciences at Fort Sam Houston in San Antonio, Texas, and as Commander of the 485th Medical Detachment, also at Fort Sam Houston.

In 1984 he enrolled in the graduate school at the University of Florida to pursue advanced studies in entomology, and shortly thereafter, he was promoted to the

rank of Lieutenant Colonel. He is a member of Alpha Zeta honorary fraternity, Gamma Sigma Delta, an academic honor society, the Entomological Society of America, and the American Registry of Professional Entomologists with certification in medical-veterinary entomology and acarology.

A sports enthusiast, Richard is a champion racquetball player, an avid football spectator, and in his spare time, he enjoys coaching youth soccer.

On completion of his Ph.D., he will continue active duty as the Assistant Executive Director of the Armed Forces Pest Management Board at Walter Reed Army Medical Center, Forest Glen, Maryland. He will be accompanied by his wife, Bonnie, and children, Rachel and Joshua.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Richard S. Patterson, Chairman
Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Philip G. Koehler
Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Frank Slansky, Jr.
Associate Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Michael D. Corbett
Professor of Food Science and Human Nutrition

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August 1987

G. L. Gabaniah
Dean, College of Agriculture

Dean, Graduate School